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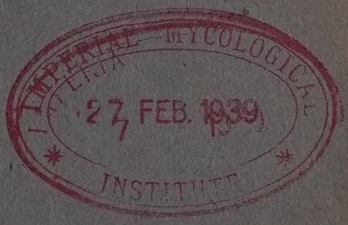

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MATHÉMATIQUES

SUR LES POLYNÔMES D'UNE VARIABLE COMPLEXE

Par V. S. FEDOROFF

(Présenté par N. Lusin, de l'Académie, le 14. VII. 1938)

Soit $f(z)$ un polynôme de degré n. Posons

$$W = f(z).$$

Si nous faisons varier z à l'intérieur d'un domaine ouvert, convexe et borné D, le point W décrit une surface riemannienne. Désignons par $S(D)$ l'aire totale de cette surface et par $|D|$ l'aire de D. Soit enfin z_0 un point situé à l'intérieur ou sur la frontière du domaine D. Cela posé, l'auteur démontre les théorèmes suivants:

I. Pour tous les polynômes de degré $n\,(n \geqslant 1)$ on a:

$$\frac{S(D)}{|D|} \geqslant \frac{|f'(z_0)|^2}{\varphi(n)}$$

où on peut toujours prendre $\varphi(n) = 2^{2n-2} \cdot n^{2n-1} \cdot (2n-1)$.

II. On peut prendre $\varphi(n) = n(2n-1)$, si les zéros de la dérivée de $f(z)$ sont extérieurs à un cercle de rayon R et le domaine D est situé dans un cercle concentrique de rayon $\frac{R}{3}$.

III. Considérons une famille de polynômes $f(z)$ tels qu'on ait:

$$f'(z) = A \cdot (z-\alpha_1)^p \cdot (z-\alpha_2)^p \ldots (z-\alpha_k)^p,$$

où $k+1 \leqslant N$, N étant une constante, la même pour tous les polynômes de la famille. A, $\alpha_1, \alpha_2, \ldots, \alpha_k$ sont des nombres complexes quelconques et p est un entier positif quelconque. Pour ces polynômes on peut prendre $\varphi(n) = 2a^2n^2$, où $a = 2^{N-1} \cdot N^{N-1}$.

IV. Si le domaine D est l'intérieur d'un cercle quelconque, on peut prendre $\varphi(n) = n^2$ (pour tout polynôme de degré n).

V. Soit D un domaine connexe limité par une frontière F, jordanienne ou non. Désignons par $\mathfrak{R}$ le rapport

$$\mathfrak{R} = \frac{\iint_D |P(z)|^2 d\sigma}{|P(z^*)|^2}$$

où $P(z)$ est un polynôme quelconque de degré n, et où z^* est un point arbitraire de la frontière F.

1) Si D est un domaine simplement connexe limité par un contour fermé simple C qui admet une tangente et une courbure continues partout, nous aurons:

$$\mathfrak{R} > \frac{K}{n^2}, \quad n = 1, 2, 3, \ldots$$

où K est une constante positive dépendante du contour C.

2) Il existe un contour fermé simple C admettant une tangente et une courbure continues partout, sauf en un point unique z_0 du contour C, et tel que l'inégalité:

$$\Re \quad K \cdot \frac{\theta^n}{n}$$

sera vérifiée pour une infinité des valeurs $n_1 < n_2 < n_3 < \ldots < n_\nu$ de n, K et θ étant des constantes positives, $\theta < 1$.

3) Quel que soit un domaine connexe D limité par une frontière quelconque F (jordanienne ou non), on a sûrement l'inégalité:

$$\Re > K \cdot \frac{\theta^n}{n},$$

où K et θ sont des constantes positives convenablement choisies, $\theta < 1$.

Soit $L(d)$ la longueur d'une courbe décrite par le point $W = f(z)$ lorsque le point z décrit un segment rectiligne d issu d'un certain point z_0. Les théorèmes suivants sont démontrés par l'auteur:

VI. Pour tout polynôme $f(z)$ de degré n on a:

$$L(d) \geqslant \frac{|f'(z_0)| \cdot |d|}{\psi(n)},$$

où $|d|$ est la longueur de d et où on peut toujours prendre $\psi(n) = 2^{n-1} \cdot n^n$.

VII. Si les zéros de la dérivée de $f(z)$ sont extérieurs au cercle $|z - z_0| \leqslant |d|$, on peut prendre

$$\psi(n) = n.$$

VIII. On peut prendre pour la famille des polynômes considérés au théorème III

$$\psi(n) = 2^{N-1} \cdot N^{N-1} \cdot n.$$

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Ivanovo.

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PHYSICS

ON SPLITTING OF NUCLEI BY COSMIC RAYS AT ALTITUDES ACCORDING TO OBSERVATIONS CONDUCTED WITH THE HELP OF PHOTOPLATES WITH A THICK LAYER

By A. GDANOW

(Communicated by W. I. Vernadsky, Member of the Academy, 10.VII.1938)

Within the last two years, besides the Wilson chamber, photoplates have also been used for studying the heavy particles of cosmic radiation.

Wilkins and Helens (1) observed a track containing about 350 grains on plates lifted into the stratosphere. This they ascribed to α-particles with an energy of 100 MeV. In the experiments of Rumbaug and Locher (2), as well as in those of Schopper (3), attempts were also made to utilize photoplates for detecting of neutrons in cosmic rays. Several interesting cases of the splitting of nuclei under the action of cosmic rays have been observed by Blau and Wambacher (4) on plates raised to an altitude of 2300 m.

In the work (5) conducted by the author together with I. I. Gourevich and A. N. Philippov a large number of cases (~10 000) of the splitting of nuclei by cosmic rays at sea level have been observed and studied by the method of thick-layer photoplates (6). Most of the splittings observed in this work appeared in the shape of trifurcations; more seldom as forks of 4 components, with only a few cases of a 5-component fork. Moreover, there are on record cases of splitting as «showers» of three or four particles with components belonging to protons and «semi-heavy» particles.

Similar cases of nuclear splittings were later observed in the Wilson chamber by Brode and Starr (7).

The author has recently carried out experiments on the study of nuclear splittings by cosmic rays at great altitudes, resort being had to thick-layer photoplates raised to the desired height on aeroplanes. The plates were raised to an altitude of 9000 m, the mean height amounting to 4500 m within 18 flying hours.

The preliminary microscopic examination of several plates (both raised to high altitudes and just left on the earth during the same time) permitted of drawing certain interesting conclusions, namely: on plates exposed at high altitudes a greater number of splittings are observable than on those placed for the whole time on the earth. A by far greater number of splitting fall to the share of forks with 5 and 4 components.

This fact may serve as a confirmation of the conclusion following from Bohr's (8) theory to the effect that with increase in energy of nuclear excitation the ejection of several particles from the nucleus becomes more probable.

It is also worth noting that among 5-component forks there are to be found, along with cases of a more or less random distribution of ejected particles, also such forks of 5 components in which 4 of the shorter components run in a beam approximately in one direction while the 5th component—a considerably longer one—runs in an exactly opposite direction. These are the so-called «tailed forks». An example of such a fork is represented in Fig. 1. The paths of particles in it when reduced to terms of air covered (counting from below upwards counter-clockwise) constitute 16, 6, 6, 4 and 5 cm. On the basis of the mean distance between the grains along the tracks it is possible to ascribe the second component to an α-particle; and the first

Fig. 1. ×330

Fig. 2. ×330

and third ones—to protons, while the fourth and fifth ones will apparently have to be ascribed to particles with weaker ionization than in the case with the protons (in Fig. 1 the ends of the first and second tracks project from the plane of focussing of the microscope while the end of the fifth track terminates a little above the fallen out lump of AgBr).

In the plates exposed at great altitude «showers» consisting of 5 and 6 particles have also been observed (the separate components in which should be ascribed both to protons and «semi-heavy» particles). In Fig. 2 an example is given of a «shower» of 5 particles with paths of 2.5, 7, 10, 5 and 9 cm of air; in this case it is only the fourth track that can be ascribed to a proton, while the others fall to the share of «semi-heavies». With the aforesaid splittings, there are all reasons to infer the existence of emission of both non-ionizing and slightly charged particles, though the phenomenon in question so far fails to be registered by the photographic method.

As regards solitary tracks, no sharp increase in their number can evidently be noticed on plates exposed at altitudes. Among them a track of about 6 m of air had so far been observed (which corresponds to the least 20 MeV in the case of a proton), while among the shorter tracks some should, as it appears, be ascribed to «semi-heavy» particles.

In the work of Taylor [9], Fraser and Dabholkar it is recorded that on plates exposed in Casmir (altitude above sea level 8000 feet, latitude 34° North) and containing a large number of lumps of fallen out AgBr, there were often detected in the vicinity of these lumps groups of solitary tracks—«clusters»—directed in a random way. In the experiments conducted by the author an increased content of lumps of AgBr in the emulsion of the plates was also observed (this being due to the fact that work with the plates was performed in warm weather), but no «clusters» were found.

Though the position of the plates in these experiments was oriented, nothing can so far be said about the preferred direction of particle emission. For the solution of this question it is necessary to perform a detailed statistical study of the material of the experiments now under way.

Radium Institute.
Academy of Sciences of the USSR.

Received
15. VII. 1938

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PHYSICS

SOFT RADIATION FROM BROMINE

By L. I. ROUSSINOW and A. A. YUSEPHOVICH

(Communicated by A. F. Joffe, Member of the Academy, 28.VII.1938)

It has been shown in a previous research carried out at the Leningrad Physico-Technical Institute (1) that in the case of bromine with its two stable isotopes three radioactive nuclei are formed upon the capture of slow neutrons, their half-lifes being 18 min., 4.2 hours and 36 hours respectively.

The above fact found explanation on the basis of a theory of nuclear isomerism advanced by Weizsäcker (2) who supposed the isomeric state to be connected with the metastable levels of the nucleus. However, this theory, when compared with experimental evidence, meets with serious difficulties. Thus, from experimental data (3), the excitation energies of the metastable levels work out to values so high that both γ-transitions and β-transformations would be forbidden owing to the necessity of assuming high values for Δl too.

When examining the radiation from the isomeric bromine nuclei decaying with half-periods of 18 min. (Br_I) and 4.2 hours (Br_{II}) (4), the latter has been found to emit a soft radiation, a fact which at once promised to throw additional light on the problem of isomerism.

Ethyl bromide was bombarded with neutrons from a (Rn+Be) source. Great care was taken during the chemical procedure to obtain a sufficiently thin layer of the radioactive substance. Target as well as counter were placed inside an evacuated vessel; the electrons penetrated into the counting space through an aperture made in the stopper of the counter, the layer by which the electrons were absorbed having 1 cm air-equivalent.

The intensity of the soft radiation component was found to decrease with a half-period equal to 4.2 hours; a cellophane filter of 0.00035 g/cm² reduced the soft radiation to half its original value, while a similar filter of 0.0008 g/cm² absorbed it completely. By applying a magnetic field it could be ascertained that the radiation consisted of slow electrons, the energy of which proved to be in close agreement with corresponding absorption data. This energy can be put equal to 30 000 eV.

The ratio of the intensity of the soft component to that of the hard component was found equal to about unity, i. e. one hard electron and one soft electron are emitted upon each disintegration.

Prof. Kourtschatow has advanced the hypothesis that, alongside of the γ-transitions and β-transformations, a transition of the nucleus from the excitation level to the normal level by way of internal conversion might also occur. Experiments carried out to check this conjecture permit of regarding it confirmed and the observed radiation can now be assumed to consist

of electrons due to the internal conversion of Br_{II} nuclei possessing excitation energies of 40 000 eV.

The study of the soft component seems to indicate that the excitation energy of the isomeric bromine nucleus is about 40 000 eV, so that, in this case, the value of Δl needs not be higher than 3.

The general reaction scheme involved is not yet known; it might be assumed that Br_I decays in the normal way, while Br_{II} possesses an excitation energy of 40 000 eV. It may be anticipated that, as a result of internal conversion, the bromine nucleus decaying with a half-period of 4.2 hours goes to the fundamental state with a subsequent β-transformation, the spectral distribution being the same as in the case of Br_I. The soft electrons may also be emitted during the internal conversion by the excited krypton nucleus succeeding the β-transformation. In the experiment, the two schemes are separable; they should be brought into accordance with the general energy balance, for which, however, no sufficient data are as yet available.

In the case of a solid target, the ratio of the activity of Br_I to that of Br_{II} remains constant, being 2 : 1. When the target is obtained in a chemical way, the above ratio varies according to the activation time from 2 : 1 to 5.3 : 1 when the activation time is increased from several minutes to 40 hours. It has also been found that Br_{II} attains equilibrium much sooner than it would follow from its half-change value. This abnormal behaviour might be due to a loss of the radioactive atoms remaining for a long time in the mother liquor, though it may be ascribed to some peculiarities of the transformations under consideration.

Physico-Technical Institute.
Leningrad.

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19. VII. 1938.

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PHYSICS

ON THE RATE OF VARIATION OF IRON MAGNETIZATION AT DIFFERENT SECTIONS OF THE HYSTERESIS LOOP

By R. V. TELESNIN

(Communicated by S. I. Wawilow, Member of the Academy, 19.VII.1938)

In the present work an installation was used which was somewhat modified as against the one used by the author in his preceding works ([1], [2]). After reiterated commutation of the current in the magnetizing winding of a very small toroid 1.25 cm in diameter made of the iron subjected to investigation, a field $H_1' = +20$ Oe (point *1* in Fig. 1) was established. Then the current was switched on in a rectilinear wire passing along the axis of the toroid which superimposed upon field H_1' an oppositely directed field, H_2, ranging from 0.5 to 4.8 Oe. Thereupon field H_1' underwent a reduction in size down to magnitude $H_1 = +0.2$ Oe and thus the transfer from point *1* to point *2*, *2'* etc. was accomplished according to the size of field—H_2 (Fig. 1). Next, Helmholz's pendulum was set in motion which cut off field H_1 and switched a ballistic galvanometer onto the winding of the toroid. This connection was made either at the moment of the disconnection of the magnetizing circuit or within a strictly fixed period of time, ranging from 1.10^{-6} to 8.10^{-3} sec. after this disconnection.

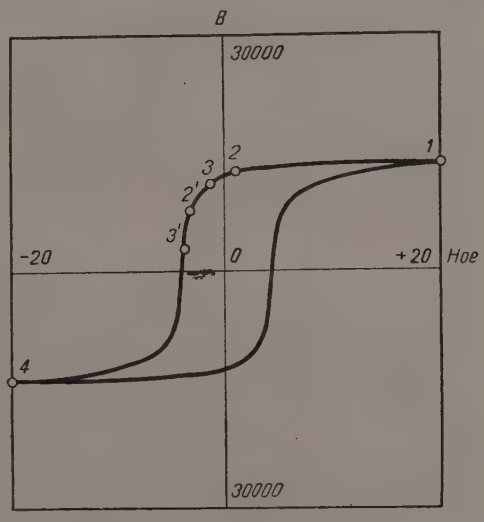

Fig. 1.

Rate of division of pendulum (1.0144—0.0085) 10^{-6} sec.

The disconnection of the magnetizing circuit transfers the iron from point *2* (*2'* etc.) to point *3* (*3'* etc.). The deflection of the galvanometer connected to the toroid for a space of time of about 0.02 sec. fixes the variation of the induction occurring in the iron after the change of the field and the connection of the galvanometer. Change of induction ΔB was calculated by the de-

flection of the galvanometer graduated in Maxwells, whereupon the mean differential permeability $\mu_d = \frac{\Delta B}{\Delta H}$ was calculated on a section of 0.2 Oe. We shall henceforward term this permeability: dynamic differential permeability $\mu_{dynam.}$ as distinct from static differential permeability $\mu_{stat.}$ determined along the hysteresis curve taken by the usual commutation method. The dynamical permeability is less than the static one, especially in the steep portion of the loop, and its maximum fails to coincide with the maximum of static permeability, being displaced by 0.2—0.3 Oe in the direction of the stronger fields. This decrease of dynamic permeability is caused by magnetic toughness and involves an expansion of the hysteresis loop. Such an expansion was observed by Lapp [3] in course of rapid processes.

The deflection of the galvanometer is approximately proportional to the change in magnetization taking place after switching in the galvanometer [4, 5]. From a number of readings obtained with different values of time, t, elapsed between the disappearance of the field H_1 and the switching in of the galvanometer, the curve of change in magnetization is plotted, while with the help of this curve we find the times of the change in magnetization up to 0.5 and 0.1 of the magnitudes of its initial change. These values serve as a measure of the duration of the process.

Field H_2 altered by 0.1 which afforded the possibility to pass the whole steep portion of the hysteresis loop in small sections. As experimental material we took toroids of annealed wire 0.0043; 0.0097; 0.0335; 0.1360 cm in diameter and bands of the same material 0.0006 cm thick. After annealing the iron was sealed off with an alkaline varnish. The mass of the iron, its cross-section and other data are shown in the table.

In columns 7—8 of this table are to be found the times of alteration of magnetization up to 0.5 and 0.1 of the magnitude of the initial change, i. e. the change occurring with the similtaneous switching in of field H_1 and the galvanometer. Columns 9—12 comprise ratios of observed time of magnetization change with maximum value μ_d to calculated time, this being done upon the assumption that the process is dependent upon the lag effect of eddy currents. This time was calculated according to Rayleigh's formula [6]:

$$t = -\frac{\ln b \, \pi r^2 \sigma \mu_d}{1.44}$$

where b is that part of magnetization change which occurs after the time moment t, σ—conductivity in $CGSM$ units, μ_d —differential permeability and r—radius of wire.

The evaluation of time t for the band was performed according to the curve of magnetization changes for a lamina [7].

Rayleigh's formula was resorted to for the sole reason that the value of t obtained from it is the greatest. Wwedensky's theory [5], though, admittedly, more relevant and, evidently, more appropriate to the true course of the process, gives smaller values for time t: for time of magnetization change only half as much and 14% less for time of change up to 0.1.

In columns 9—10 $t_{calc.}$ was calculated according to the values of static permeability $\mu_{stat.}$, while in columns 11—12 this reckoning was made according to dynamical permeability $\mu_{dynam.}$ Column 13 shows the values of magnitude $\frac{t_{0.1\ obs.}}{\mu_{dynam.} d^2}$ which in the case of a process caused by eddy currents should be constant. The outstanding difference between the values of this magnitude for thick and thin wires shows most clearly that eddy currents are not responsible for the observed long duration of the processes of the magnetization change in thin wires.

d, cm	S_1, cm² (of the whole iron)	S_2, cm² (of one wire)	p_r	μstat. maxim.	μdynam. maxim.	Observed		Calculated according to				$t_{0.1}$ obs. / μdynam. d^2
						$t_{0.5} \cdot 10^{-6}$ sec. max.	$t_{0.1} \cdot 10^{6}$ sec. max.	μstat.		μdynam.		
								$t_{0.5\,obs.}$ / $t_{0.5\,calc.}$	$t_{0.1\,obs.}$ / $t_{0.1\,calc.}$	$t_{0.5\,obs.}$ / $t_{0.5\,calc.}$	$t_{0.1\,obs.}$ / $t_{0.1\,calc.}$	
1	2	3	4	5	6	7	8	9	10	11	12	13
0.0006	0.0032	—	0.1	18400	9460	290	2410	188	313	207	344	0.707
0.0043	0.00317	$14.5 \cdot 10^{-6}$	0.1	41600	13800	645	5200	55	133	96	231	0.0204
0.0097	0.01030	$73.8 \cdot 10^{-6}$	0.32	31400	11300	1155	4220	5.22	19	12.8	46.8	0.00396
0.0335	0.00855	$880 \cdot 10^{-6}$	0.26	6600	2185	356	1930	2.08	3.86	5.47	8.95	0.000787
0.1360	0.0145.	$14500 \cdot 10^{-6}$	0.445	7500	2750	1840	7440	0.66	0.8	1.365	1.67	0.000146

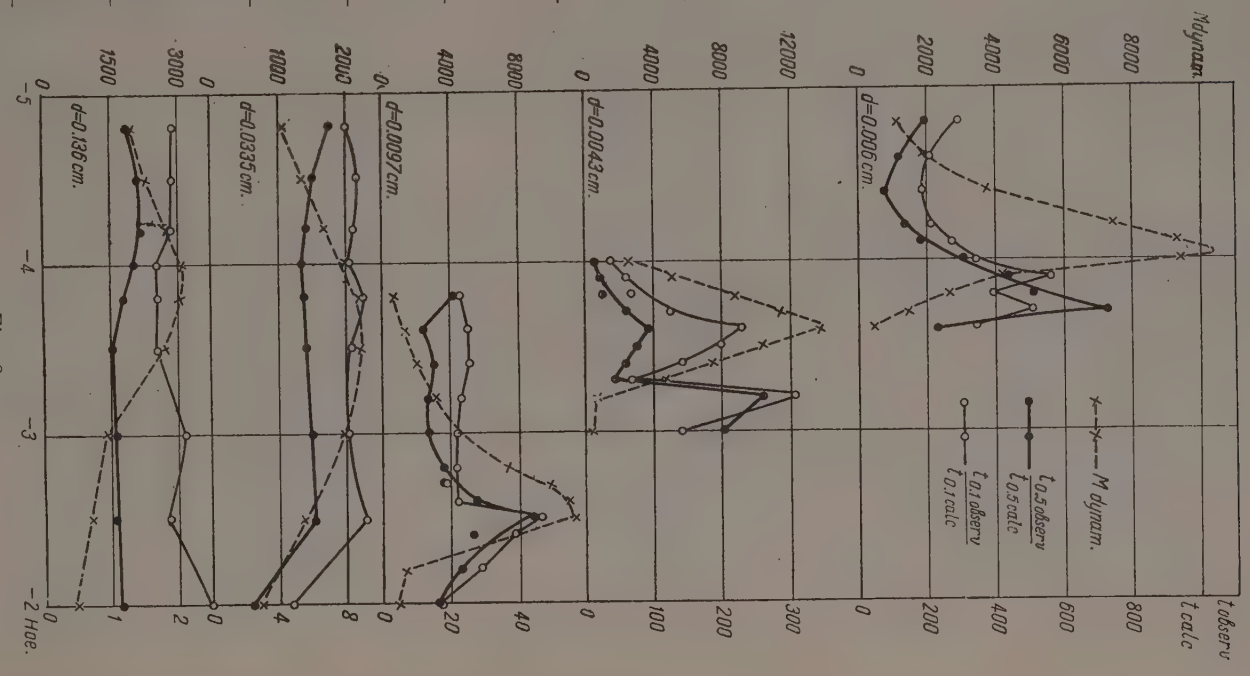

Fig. 2.

From the data of the table it will be readily seen that in thick iron (0.136 cm) the duration of the process of magnetization change may be largely ascribed to the action of eddy currents whose development is determined by dynamical permeability. Should we, however, assume that the action of eddy currents is to be determined by static permeability, then the calculated values of time t (columns 9—10 of the table) ought to exceed the observed values of time which is, of course, impossible. This fact thus leads direct to the conclusion that the effect of eddy currents is determined by dynamic permeability.

For all other wires and bands, and particularly for the thinnest ones, the observed time of magnetization change exceeds many times (up to 732!) the calculated figure, which fact points to the impossibility of accounting for the observed long duration of the process by the effect of eddy currents. Obviously, the slow progress of the process is here called forth by magnetic viscosity. Fig. 2 illustrates the curves of dynamic permeability and the ratio $\frac{t_{obs.}}{t_{calc.}}$.

The maximum of this ratio coincides on most curves with the maximum of permeability as well as with the maximum of duration of the process of magnetization change. This shows that magnetic viscosity, just as eddy currents, depends upon μ_d though in respect of viscosity the dependence is more complicated. This conclusion is in good agreement with the results obtained by Kühlewein [8] with permalloy and those of A. V. Mitkevich [9] with an iron wire.

In order to become confident that induction currents are not responsible for the long duration of the process in a rectilinear wire, the current in which is set up by field H_2, another wire connected to a very small resistance was introduced beside the mentioned one. With the magnitude of the resistance equalling 0.1 Ω, there could be observed no difference in the duration of the process. Only with a resistance of the order of 0.01 Ω did the duration of the process increase 10% with a change of the field by $\Delta H = 0.4$ Oe. The resistance of the circuit itself was from 0.5 to 4 Ω.

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PHYSICS

THE SPECTRUM OF VISIBLE RADIATION PRODUCED BY FAST ELECTRONS

By P. A. CHERENKOV

(Communicated by S. I. Wawilow, Member of the Academy, 21.VII.1938)

1. As far back as at the earliest stage (1) of the study of the visible radiation of pure liquids produced by fast electrons it was found by us that the energy of this radiation increases in the direction of short wave lengths. In later experiments (2) an approximate evaluation was made of the relative energies emitted within a certain small range of wave lengths for parts of the spectrum lying in the vicinity of $\lambda = 444$ mμ and $\lambda = 522$ mμ; in course of these investigations it was found that in the first case ($\lambda = 444$ mμ) the measu-

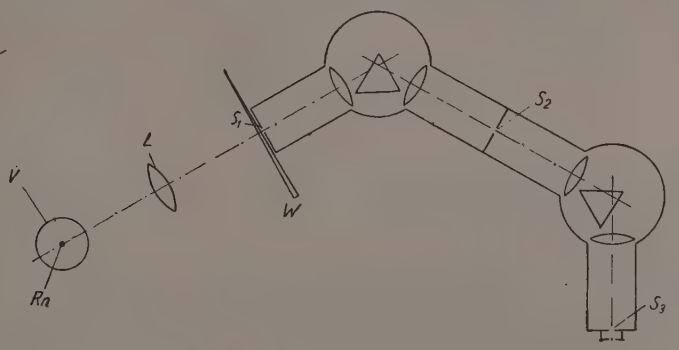

Fig. 1.

red intensity of luminosity was 1.5—2 times as great as in the second case ($\lambda = 522$ mμ).

For sake of comparison with theory (3) it appears of interest to investigate in detail the dependence of emitted energy upon wave length.

2. Observations were effected in the visible part of the spectrum with the help of a double glass monochromator. At a distance of 45 cm from the entrance slit of this monochromator (Fig. 1) was placed a thin-walled glass vessel, V, for the liquid. The vessel was cylindrical in shape and measured 3 cm in diameter and 4.5 cm in height. As the source of emission of fast electrons was used a glass tube with radium emanation suspended

on a thin wire inside vessel V, along its axis. The cross-section of this tube was 2 mm while the thickness of its walls equalled about 0.1 mm. The amount of emanation in the different series of experiments varied from 100 to 280 mC. In order to eliminate the luminescence of the glass of the tube, its outside surface was coated with silver. Under these conditions the luminescence of the liquid in vessel V was brought about principally by the β-rays of Ra (B+C), while only an inconsiderable part of this luminosity was called forth by the recoil-electrons formed in course of the Compton scattering of γ-rays.

The luminous volume of the liquid taking the shape of a vertical cylindrical column (8—10 mm in cross-section) was projected in its natural size on the entrance slit (S_1) of the monochromator by means of lens L. The due part of the spectrum was brought out by slit S_2; the eye of the observer had its position directly outside the exit slit S_3. All the measurements were carried out by the quenching method ([4]). Outside entrance slit S_1 a neutral grey wedge was placed whose function consisted in diminishing light intensity

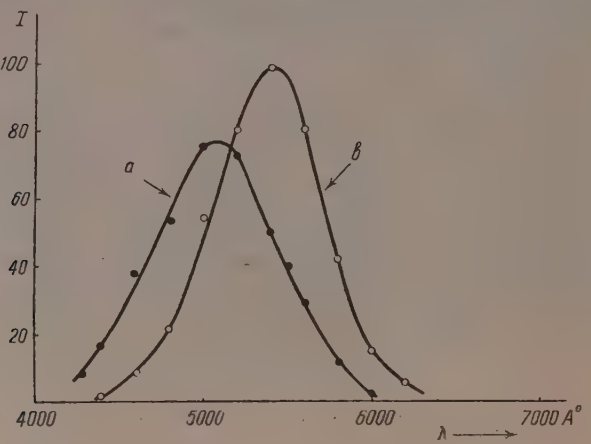

Fig. 2.

down to the threshold of the visual excitation of the eye. The shifting of this wedge during the process of measurements was accomplished by the assistant of the investigator.

As a light source with a given spectral distribution of energy use was made of a Heffner lamp whose brightness was also measured after the sight threshold. In these measurements, instead of throwing light directly on the entrance slit, the Heffner lamp was made to illuminate a plate coated with magnesium oxide and located in place of vessel V. All the other conditions were the same as in the measurements of luminosity called forth by fast electrons.

Owing to the fact that brightness $I(\lambda)$ observed in measurements after the quenching method is proportional to the eye sensitivity $f(\lambda)$ multiplied by the energy $E(\lambda)$ emitted from the light source within the given region of the spectrum, the energy of the luminosity under consideration (for that region of the spectrum) will be determined by the expression

$$E(\lambda) = E_1(\lambda) \cdot \frac{I(\lambda)}{I_1(\lambda)}, \qquad (1)$$

where $I_1(\lambda)$ is the observed brightness and $E_1(\lambda)$—energy of radiation of the Heffner lamp.

3. The measurements of the brightness of luminosity produced by the effect of β-particles of Ra (B+C) were effected for benzol. Three series of observations were carried out (on different days), the range under observation extending from $\lambda = 430$ mμ to $\lambda = 600$ mμ. Three series of observations were also performed for the luminosity of the Heffner lamp ranging from $\lambda = 440$ mμ to $\lambda = 620$ mμ. The results of all 3 separate series were found to coincide, this being true both for the case of benzene radiation and for that of the radiation of the Heffner lamp. The breadth of the slits of the monochromator was taken in dependence upon the amount of radium emanation and was equal either to 0.3 mm or to 0.5 mm. The respective breadth of the spectral interval selected was in the first case 27 Å for a mean wave length of 440 mμ and 90 Å for a mean wave length of 600 mμ. In the second case (slit equalling 0.5 mm) the breadth of the part of the spectrum covered rose to 45 and 150 Å respectively. As evidenced by measurements, the change in the breadth of the slits from 0.3 to 0.5 mm had no marked effect upon the final results. The numerical results of the measurements reduced to terms of equal emanation and equal breadth of slit are presented in Tables 1 and 2 (the first three lines).

Table 1

Radiation of Benzene under the Action of β-Rays

Wave length (in mμ) / Observed brightness (in relative units)	430	440	460	480	500	520	540	550	560	580	600
1st series of observations . .	8.5	15.5	37,5	46.5	75.5	66.0	—	40	—	11.5	—
2nd » » » . .	—	19.2	37.8	61.6	72.2	70.0	51.8	—	33.6	12.9	—
3rd » » » . .	—	14.8	38.3	54.0	77.9	81.9	48.2	—	24.8	11.0	2.5
Mean $[I(\lambda)]$	8.5	16.5	37.9	54.0	75.2	72.6	50.0	40	29.2	11.8	2.5
$E(\lambda) = E_1(\lambda) \frac{I(\lambda)}{I_1(\lambda)}$	—	68.1	55.0	50.2	42.3	40.5	33.0	—	31.5	32.6	25.5
$1/\lambda^3 \cdot 10^{11}$	—	1.175	1.026	0.9	0.8	0.704	0.633	—	0.568	0.51	0.463

Table 2

Radiation of Heffner Lamp

Wave length (in mμ) / Observed brightness (in relative units)	440	460	480	500	520	540	560	580	600	620
1st series of observations	—	9.8	23.8	72.6	73.6	84.4	—	47.4	15.4	5.8
2nd » » »	1.82	8.5	20.8	43.8	77.5	102.5	87.5	44.5	15.8	6.1
3rd » » »	1.62	7.2	19.4	47.0	89.8	108.0	73.0	36.0	13.8	5.2
Mean $[I_1(\lambda)]$	1.72	8.5	21.3	54.4	80.3	98.3	80.3	42.6	15.0	5.7
$E_1(\lambda)$ (according to Pearson and Pleasance's data)	7.1 (extrap.)	12.3	19.9	30.5	44.4	64.9	86.5	117.6	152.6	—

The mean results of each of these tables are plotted in Fig. 2. In this figure curve *a* corresponds to the radiation of benzene, curve *b*—to that of the Heffner lamp. Making good use of the data obtained by A. R. Pearson and B. Pleasance (5) on the spectrum distribution of energy in the flame of a

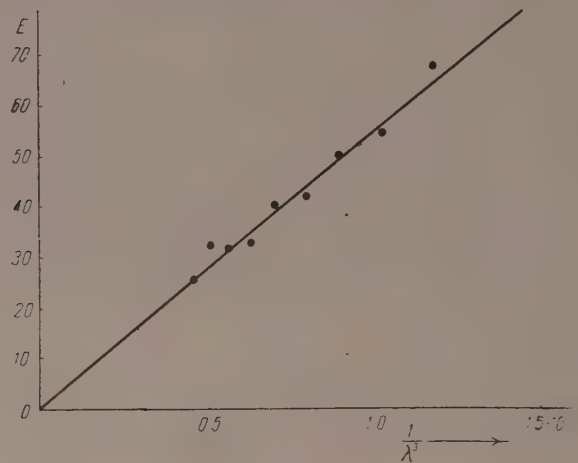

Fig. 3.

Heffner candle, it is possible from the points of these curves and according to formula (1) to calculate the values of $E(\lambda)$. These values (in relative units) are recorded to line five of Table 1. The figures of this line show that the energy of radiation produced in benzene by fast electrons grows uniformly in the direction of short wave lengths. The only exception represented

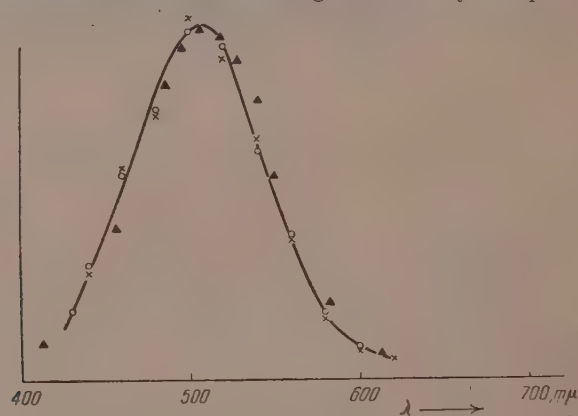

Fig. 4. Curve of visibility with twilight vision.

by the value of $E(\lambda)$ for $\lambda = 580$ mμ lies within the limits of experimental error.

In Tamm and Frank's (3) theory the emission energy of a single electron in the phenomenon under consideration is proportional to the expression:

$$\int \nu d\nu \left(1 - \frac{1}{\beta^2 n^2}\right).$$

In view of the fact that in the investigated region of the spectrum the index of refraction n may be considered independent of wave length, the inference can be made from the expression above referred to that the emission energy should vary in proportion to $\frac{1}{\lambda^3}$.

The graph of Fig. 3 shows that the linear relationship between $E(\lambda)$ and $\frac{1}{\lambda^3}$, imposed by Tamm-Frank's theory, is indeed fulfilled.

If the data of Tables 1 and 2 be reduced to the terms of a spectrum with uniform distribution of energy (with due account taken of the dispersion of the monochromator), then, as a collateral result of our investigations, it will be possible to obtain a curve characterizing the spectrum sensitivity of the eye (the author's) for twilight vision. This curve is plotted in Fig. 4. The crosses in this curve correspond to observations with the Heffner lamp, and the circles—to observations with the radiation of benzene under the effect of β-particles, it being assumed in the latter case that the emission energy varies in inverse proportion to the third power of wave length. The inconsiderable divergence of the curve of the sensitivity of the author's eye from the results of other measurements ([6]) (the latter are represented in the graph by triangles) can be accounted for by individual differences of the eye with different observers.

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PHYSICS

THE ANOMALOUS MAGNETIC PROPERTIES OF ANHYDROUS $FeCl_2$

By S. S. SHALYT

(Communicated by S. I. Wawilow, Member of the Academy, 21.VII. 1938)

The investigation of the magnetic properties of the anhydrous salt $FeCl_2$ at low temperatures has revealed facts for which there is at present no theoretical explanation.

It has already been reported (4) that the group of anhydrous salts $FeCl_2$, $CoCl_2$, $NiCl_2$ and $CrCl_3$ shows, aside from the field dependence of susceptibility discovered in Leiden (1), another ferromagnetic property—that of hysteresis; the experimental results refer only to the residual moments of these salts at liquid hydrogen and helium temperatures.

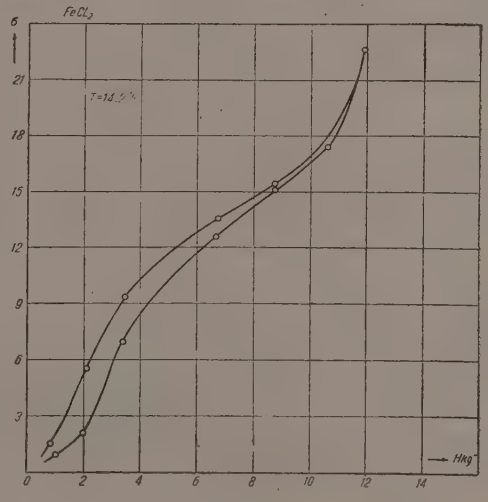

Fig. 1.

In the present paper we give the hysteresis loop for $FeCl_2$ at 14.2° K (Fig. 1). This temperature lies below the Curie point (23.5° K for $FeCl_2$), i. e. the point at which a specific heat jump was found for the group of salts mentioned above (2). The hysteresis appears at the Curie point, and becomes more marked with further reduction of temperature. In addition to hysteresis the magnetization curve $\sigma = f(H)$ shows an even more peculiar feature, namely an undulating change of magnetic moment with field, as has previously been found only in the case of diamagnetic bismuth (3). To bring out this effect more clearly we plot $\frac{\sigma}{H} = \chi$ as a function of field (Fig. 2). The resulting curve disagrees with the results of Woltjer and Wiersma (1) obtained for this salt, but this disagreement may probably be ascribed to the use these authors made of the Gouy method of measurement, which does evidently not suit with a substance whose susceptibility is strongly field dependent; our results were obtained by the more appropriate Faraday method. At higher temperatures (from 58 to 112° K) the suscep-

tibility was found to obey the Curie-Weiss law, $\chi\,(T-\Theta)=C$ with $\Theta=20.3°$ K and $C=0.0317$ (Fig. 3), in agreement with the results of Woltjer and Wiersma.

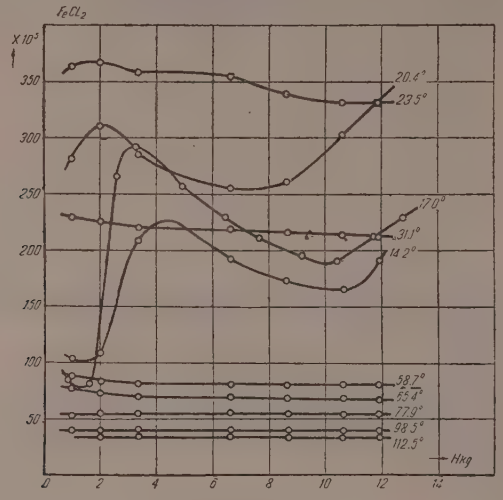

Fig. 2.

In the top left hand corner of Fig. 3 we give the temperature dependence of χ near the Curie point. The maximum of this curve shifts with increasing

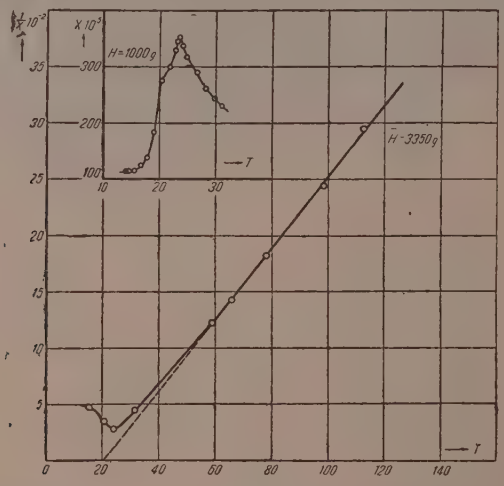

Fig. 3.

field strength towards lower temperatures, while for weak fields it coincides approximately with the temperature of the specific heat jump.

An investigation of $FeCl_2$ in very strong magnetic fields would evidently be of interest.

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GEOPHYSICS

THE INSTABILITY OF THE OZONE LAYER AT SUNRISE AND SUNSET AND THE PROBLEM OF DETERMINATION OF THE HEIGHT OF THE OZONE LAYER

By I. KHVOSTIKOV and N. ERSHOVA

(Communicated by O. J. Schmidt, Member of the Academy, 27.VI. 1938)

1. In the present communication some experiments are described and considerations given which show that the problem of distribution of the atmospheric ozone with the height seems to require an essential revision.

As is known, for computing the dependence of ozone concentration on height use is made of observations of the so-called Umkehr-Effekt, observable in the ultra-violet part of the spectrum of the dispersed light of the sky.

However, the data obtained by Rodionov, Pavlova and Stoopnikov (1) as the result of their observations carried out on high levels (during the alpine expedition) in the summer of 1937 show that the curve with the minimum is valid not only for the dispersed light, but also for direct sun rays (for $\log \frac{I_{\lambda_1}}{I_{\lambda_2}}$), the position of the minimum being approximately the same.

Whereas the explanation of the Umkehr-Effekt given by Dobson and Götz is quite acceptable in the case of the dispersed light of the sky, it does not hold in the case of direct sun rays. We have either to assume that the ozone content varies, or that a large (anomalous) refraction of the sun rays with smaller wave lengths takes place. The assumption that the ozone content between sunrise and sunset differs must be rejected, since nocturnal observations show that it is about the same at night as in the day-time.

Thus, to explain the effect observed by Rodionov, Pavlova and Stoopnikov there is nothing left but to refer it, as these authors do, to anomalous refraction (1).

However, a preliminary computation carried out by one of the authors of the present communication shows that if the anomalous refraction of ultra-violet rays in the atmosphere be considered as the result of an anomalous dispersion in the neighbourhood of the absorption layer [it seems that such phenomena must occur in the atmosphere (2, 3)], then the ozone, in virtue of its scarcity, can have no influence on the index of refraction. The course of the phenomenon must be determined by the absorption of oxygen, which lies within the range of much smaller wave lengths (2000 Å and less), and can produce no discernable difference in the index of refraction for so closely selected wave lenghts as those (λ_1=2950, 3021, 3125 and 3260 Å),

as observed by Rodionov, Pavlova and Stoopnikov. On the other hand, the question of the anomalous refraction in this part of the spectrum may be decided by direct experiment too. If the refraction for λ_1=3021, λ_2=3125 and λ_3=3260 Å is not the same and for λ_1 and λ_2 it is greater than for λ_3, then, photographing the sun's disk with a camera fitted with a quartz object-lens and light filters letting through only the light of the wave length λ_1, λ_2 and λ_3 respectivély, we should obtain different apparent position of the sun in the sky at one and the same time (for λ_1 and λ_2 a higher position than for λ_3). At present, owing to the impossibility of producing the necessary light filters, the experiment is not realizable in this form, but it can be easily carried out in a somewhat different, though equally convincing manner.

2. Let us focus the sun's disk by a quartz object-lens on the slit of a quartz spectrograph in such a way that the image of the disk d (Fig. 1) occupies only a part of the slit length S. Let d be the image of the sun's disk by the rays of wave length λ_3.

If the refraction of the rays λ_1 is greater than the refraction of the rays λ_3, then the image of the sun's disk by the rays λ_1 will occupy a different position d' on the slit. The task consists in photographing by the described method the sun rays' spectrum at zenith distances of 75—90°: if there is a greater refraction for rays with smaller wave length, then on the short wave side there must be a bending of the spectrum. The magnitude of the bend may serve for an accurate determination of the magnitude of refraction, since the height of the spectrum will obviously correspond to an angle of 0.5 (the angular dimension of the sun).

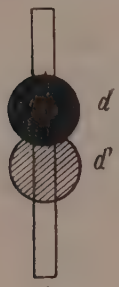

Fig. 1.

The photographing of the ultra-violet spectrum of the sun rays at zenith distances of the order of 80—90° presents the following difficulty: owing to an unproportionally strong absorption of the ultra-violet rays (compared with visible rays) at great zenith distances of the sun, an exposition of such length is required that the visible spectrum gets highly over-exposed, and the presence of an excessive amount of dispersed visible light makes the spectrograms useless for photometrical purposes. In such cases, therefore, double spectral decomposition is applied (f. i. by Dobson's spectrographs) which, however, excludes the possibility of observing the apparent position of the sun's disk and disturbs the described scheme of the experiment.

Considering the above-said, we photographed the ultra-violet spectrum of the sun rays under all the conditions necessary for the success of the described simple scheme. This was carried out in June 1938 by means of Hilger's spectrograph (F : 3) in Moscow and at a station 40 km from Moscow (Zelionaya Sloboda). The obtained spectrograms show that for zenith distances of the sun within 87 to 75° the phenomenon of additional refraction is absent within the range from 3050 to 3500 Å (the accuracy of our refraction measurements was 10′). Thus, it can be definitely asserted that the phenomenon observed by Rodionov, Pavlova and Stoopnikov for λ=3021, 3125 and 3260 Å cannot be explained by an anomalous refraction.

3. The extremal shape of the curve for $\log \frac{I_{\lambda_1}}{I_{\lambda_2}}$ for the dispersed light and for direct sun rays may be explained in a quite simple and natural way. The equilibrium of the ozone layer is determined among others by the following reactions:

$$O_2 + h\nu_1 \rightarrow O + O, \quad (1)$$

$$O_3 + h\nu_2 \rightarrow O_2 + O. \quad (2)$$

Reaction (1) is, as we know, a necessary condition for the formation of the ozone, while reaction (2) leads to a loss in ozone. The frequency ν_1 corresponds to the part of the spectrum $\lambda \leqslant 1850$ Å and ν_2 to the region from 2200 to 2900 Å.

What happens, if the intensity of the sun rays of frequency ν_1 decreases while the intensity of those of frequency ν_2 remains constant? This will lead to a loss in ozone. But if we calculate at what height the maximum absorption of the rays ν_1 (absorption by oxygen) is attained, we find the known formula of Chapman:

$$h_m = H \ln A \rho_0 \frac{H}{\cos x} ,$$

where H is the reduced height of the atmosphere, A—the absorption coefficient of the light of frequency ν_1, ρ_0—the density at the earth surface and x—the zenith distance of the sun. If x increases, h_m also increases. This means that as the sun sets, the layer of effective absorption of the rays by oxygen

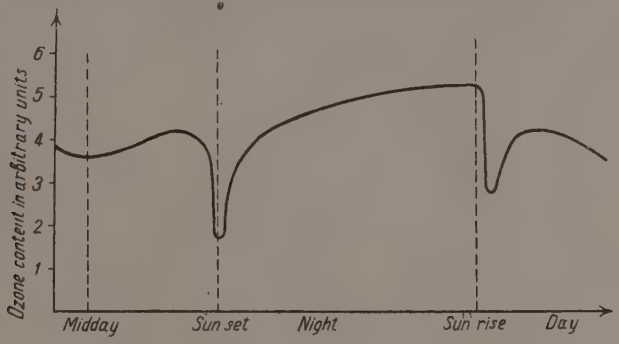

Fig. 2.

may happen to be considerably higher than the ozone layer: the light rays of frequency ν_1 will arrive at the heights where the ozone is formed (the ozone layer) in a weakened condition. But in view of the concentrated distribution of the ozone with height, the region of maximum absorption of ν_2-light by the ozone will preserve its height, namely, the height of the ozone layer. In other words, the light of frequency ν_2 will continue to be absorbed mainly in the ozone layer and will destroy the ozone molecules at the same rate. This will result in a decrease of ozone content.

The loss in ozone is, however, only temporary. After the sunset when the rays of frequency ν_2, which destroyed the ozone molecules, cease to act, the ozone content must again increase on account of the recombination of the oxygen molecules O_2 with the atoms O which remain in the atmosphere in a certain proportion even after the sunset. Ultimately the ozone content may at night not only attain its day value, but even exceed it. The presence of ultra-violet sun rays in the atmosphere is a necessary condition for the existence of atmospheric ozone (these rays produce atomic oxygen by dissociation), however, the conditions prevailing at the day-time (ultra-violet rays in the atmosphere) are, atomic oxygen being already present, less favourable for the ozone than the night conditions: the destructive influence of the rays of frequency ν_2 prevails over the influence of the ν_1 rays, favourable for the formation of the ozone. This is directly confirmed by the fact that the minimum of the ozone content falls at the midday. Thus, the mentioned loss in ozone occurring at great zenith distances of the sun is compensated after the sunset.

The daily course of the ozone content which may be expected in virtue of the above considerations is given in Fig. 2.

The temporary loss in ozone between the sunrise and the sunset may equally well account for the Umkehr-Effekt and the effect observed by Rodionov, Pavlova and Stoopnikov.

However, in this case the current interpretation of the Umkehr-Effekt proves to be insufficient, which may require a revision of the methods and the results of determination of the ozone content at different heights of the atmosphere.

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CHEMIE

FESTE LÖSUNGEN DER ORTHOSILIKATE VON CALCIUM UND BARIUM

Von N. A. TOROPOW und P. F. KONOWALOW

(Vorgelegt von F. I. Loewinson-Lessing, Mitglied der Akademie, d. 2.VII. 1938)

Die Silikate von Calcium und Barium sind zum Teil von P. Escola (1) untersucht worden. Es wurden das Diagramm der Schmelzbarkeit und die kristallinische Phasen des Metasilikatsystems $CaO \cdot SiO_2$—$BaO \cdot SiO_2$ untersucht.

In diesem System entdeckte man eine binäre Verbindung von $2CaO \cdot BaO \cdot 3SiO_2$, die bei $1320 \pm 4°$ inkongruent schmelzt. Diese Verbindung kristallisiert sich in hexagonalen Fasern mit prismatischer Spaltbarkeit. Die Lichtbrechung $N_g = 1.681$, $N_p = 1.668$.

Wir studierten das binäre Orthosilikatsystem: Orthosilikat Barium—Orthosilikat Calcium. Die Präparate wurden durch sorgfältiges Mischen von kohlensaurem Barium (Kahlbaum), kohlensaurem Calcium (reaktiv) und Quarz (Bergkristall) hergestellt (eine Vaporisation mit Fluorwasserstoffsäure ergab einen Rest von 0.15%).

Aus den Mischungen wurden kleine Zylinder gepresst und in Knallgasflammen geschmolzen. Die gewonnenen Legierungen wurden in Immersionspräparaten mikroskopisch untersucht und ihr spezifisches Gewicht wurde mit Hilfe eines Piknometers festgestellt.

Der erste und bedeutendste Moment unserer Untersuchung besteht in der Feststellung einer völligen Homogenität aller unserer Präparate.

Die mikroskopischen Untersuchungen zeigten bei allen Präparaten vollständige mikroskopische Homogenität und Einphasigkeit. Die Lichtbrechung der gewonnenen homogenen gemischten Kristalle stieg beständig mit Zunahme der Konzentration des Bariumsilikats in festen Lösungen.

Ferner wurden genaue Messungen der Lichtbrechung der gewonnenen festen Lösungen durchgeführt. Die Resultate sind in der Tabelle angegeben.

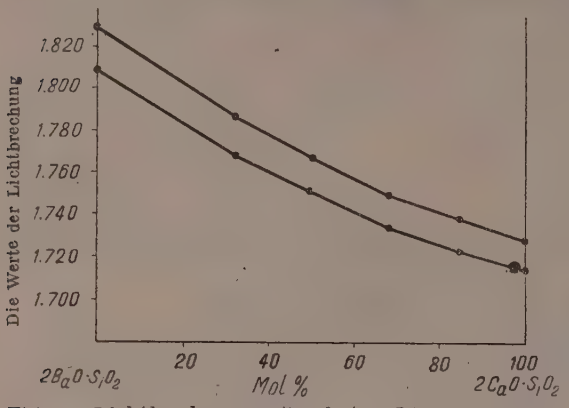
Es ist bezeichnend, dass Orthosilikat Calcium in unseren festen Lösungen in seiner hohen Temperaturform (α oder β) beteiligt ist.

Für die spezifischen Gewichte, die mit grosser Vorsicht durch Anwendung von Petroleum ermittelt wurden, da Barium und Calciumorthosilikate sich in Wasser auflösen, wie auch für die Lichtbrechungswerte, beo-

Die Werte der Lichtbrechung und der spezifischen Gewichte fester Lösungen $2CaO.SiO_2$ und $2BaO.SiO_2$.

Gewicht % $2CaO.SiO_2$	100	90	70	50	30	15	0
Gewicht % $2BaO.SiO_2$	0	10	30	50	70	85	100
Mol % $2CaO.SiO_2$	100	95.1	83.2	68.3	48.2	26.6	0
Mol % $2BaO.SiO_2$	0	4.9	16.8	31.7	51.8	73.4	100
N_{gNa}	1.735	1.737	1.720	1.757	1.763	1.783	1.830
N_{pNa}	1.717	1.719	1.725	1.737	1.749	1.768	1.810
Spez. Gewicht $d_{25°}^{4°}$	3.28	3.51	3.83	4.18	4.4	4.8	5.2

bachteten wir, dass mit der Veränderung der Konzentration der Komponente in den zu untersuchenden Präparaten eine allmähliche Veränderung der erwähnten physikalischen Grössen eintritt.



Fir. 1. Lichtbrechungswerte fester Lösungen von $2\,CaO \cdot SiO_2$ und $2\,BaO \cdot SiO_2$.

Diese Tatsache, in Zusammenhang mit der oben erwähnten Homogenität der Orthosilikatlegierungen, ist ein genügender Beweis, dass in der Orthosilikatenreihe der Elemente von Barium — Calcium eine ununterbrochene Reihenfolge fester Lösungen anwesend ist. Abb. 1 und 2 weisen entsprechende graphische Darstellungen auf.

Bisher finden wir in der Literatur die Beschreibung nur eines Falles der Entstehung einer ununterbrochenen Reihe fester Lösungen zwischen Orthosilikat Calcium und Orthosilikat eines

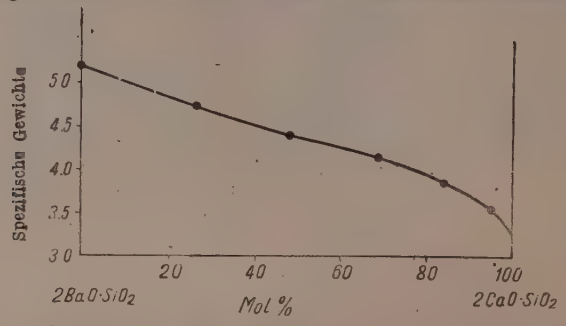


Fig. 2. Spezifische Gewichte fester Lösungen von $2\,BaO \cdot SiO_2$ und $2\,CaO \cdot SiO_2$.

anderen Metalls (Mn^{++}) ([2], [3]). In den anderen Fällen von Lithium ([4]), Natrium ([5]), Magnesium ([6]), zweiwertigem Eisen ([7]), wurde entweder die Entstehung chemischer Verbindungen, z. B. von $NaCaSiO_4$ ([5]) oder $CaMgSiO_4$ ([6]), oder fester Lösungen von beschrenkter Konzentration festgestellt.

In Anbetracht des ausschliesslichen technischen Wertes des Orthosilikats Calcium, ist es von wesentlichem Interesse einen Stoff zu gewinnen, dessen Beschaffenheit die Mitte zwischen den Eigenschaften dieses Silikats und des Orthosilikats Barium hält. Die Arbeit wird in dieser Richtung fortgeführt.

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ZITIERTE LITERATUR

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CHEMISTRY

THE ALUMINOTHERMIC METHOD OF PRODUCING CHROME-ALUMINIUM ALLOYS

By I. I. KORNILOV and V. S. MIKHEJEV

(Communicated by N. S. Kurnakov, Member of the Academy, 28.VI.1938)

During the last few years the production of new heat-resisting steels containing chromium and aluminium has been mastered in industry (1). Being of high electrical resistance, these steels can replace nichrome in electrically heated ovens and apparatus. The alloys of this type used at present in technics are of the following composition: «chromal» contains 30 per cent Cr and 5 per cent Al; «fechral» contains 13 per cent Cr and 5 per cent Al. Besides there are generally other admixtures in these alloys, viz., silicon, manganese, up to 0.5—0.6 per cent, and carbon up to 0.2 per cent.

According to practical workers, the presence of the latter element in quantities from 0.15 to 0.20 per cent tends to lower the quality of the steel and greatly deteriorates the mechanical treatment and the cold drawing of these materials. For this reason the carbon content in foreign makes of chrome-aluminium steels is brought down to the minimum, 0.03—0.04 per cent.

The normal way of getting rid of carbon would be to use pure metals in fusing. However, this method is of no practical interest, since producing pure chromium and introducing it into the alloys in its pure form is extremely difficult, and secondly because aluminium is impossible to introduce into steel normally so as to get a homogeneous structure, on account of its low specific weight and high oxidability at high temperatures.

In studying equilibrium diagram of alloys of the ternary system iron-chromium-aluminium we also faced the necessity of preparing alloys which did not contain foreign admixtures and of working out a more convenient and simple procedure of preparing these alloys. Taking into account the requirements of industry, which is interested in the preparation of alloys of iron-chromium-aluminium free from carbon, we conducted a set of experiments for finding the conditions under which we could obtain intermediate alloys and alloying elements, using which the alloys needed could be prepared in any concentration. Of the three components, chromium, aluminium and iron, it is most convenient to prepare alloying elements of chromium and aluminium, since introducing these components into the alloy in their pure form is quite difficult.

One of the easiest method of producing such metals as chromium and manganese is the aluminothermiic one, i. e. reducing the oxides of these metals with pulverized aluminium.

Unlike the methods mentioned of producing these metals after Goldschmidt's method [well described in literature (²) and (³)], we made it our purpose to produce not pure chromium, but an alloy of chromium and aluminium of a given percentage composition by calculating the charge accordingly.

The charge for producing the reaction was calculated as follows: aluminium was added in such quantity that after the chromium oxide was reduced according to the reaction

$$Cr_2O_3 + 2Al = 2Cr + Al_2O_3 + 104 \text{ cal. of heat,}$$

the excess would immediately fuse with the chromium, forming an alloy of the concentration calculated.

Using pure materials to begin with aluminium (containing 0.15—0.2 per cent Si) and pure chromium oxide (Soyuzlaborreactiv), we prepared a charge of the necessary composition and conducted the reaction in Chamotte crucibles. Lest the aluminium reduce the silica of the crucible, the walls of the latter were smeared with a layer of aluminium oxide with aluminium chloride as the fixer. The capacity of the crucible was such that up to 800—1000 g of charge could be prepared, with an end to produce up to 400—500 g of alloy.

The charge, consisting of chromium oxide, powdered aluminium and potassium chlorate (to speed up the reaction), was carefully mixed, put into the crucible and pounded. Then an ignition mixture of 1 g powdered Mg and 8 g BaO_2 was put into a little hole made in the middle of the charge. A magnesium ribbon was stuck into the ignition mixture in order to light it. When the reaction was over (it lasted 45—60 sec.) and the crucible had cooled off, the alloy was separated from the slag, weighed and analyzed. All the alloys produced were perfectly homogeneous and small-grained, but brittle, so they easily broke into pieces upon being hit with a hammer.

On this manner we prepared altogether 7 alloys of chrome-aluminium and 3 alloys of iron-chrome-aluminium, the composition of which are given in the table. The latter also gives the results of chemical analysis, the aggregate percentage yields of the alloying element and the yield of chromium and aluminium separately.

№	Weighed portion of alloy, g calculated	Percentage composition according to synthesis			Percentage composition of alloy according to analysis				Total weight produced in g	Yield in %		
		Cr	Al	Fe	Cr	Al	Fe	Si		Total	Cr	Al
1	473	95	5	—	95.90	3.27	—	0.83	401	84.67	85.54	55.37
2	500	85	15	—	83.50	16.00	—	0.50	451	90.60	84.06	90.18
3	492	80	20	—	82.04	17.75	—	0.39	449.5	91.36	90.00	81.00
4	520	77	23	—	77.54	22.46	—	—	460	88.47	89.17	86.08
5	361	75	25	—	73.82	25.68	—	0.50	324.50	89.89	78.02	95.69
6	500	70	30	—	77.54	22.16	—	0.30	462	88.6	89.17	86.08
7	400	65	35	—	67.39	32.19	—	0.42	300	75.00	77.76	68.96
8	279.99	43.26	14.48	43.26	33.97	14.41	51.62	1.00	235	85.77	67.35	86.60
9	272.56	41.27	17.46	41.27	33.97	17.55	48.48	0.40	216.80	79.54	73.45	80.00
10	500.00	41.70	17.60	41.70	36.99	14.65	47.80	0.56	476.00	95.2	84.74	82.62

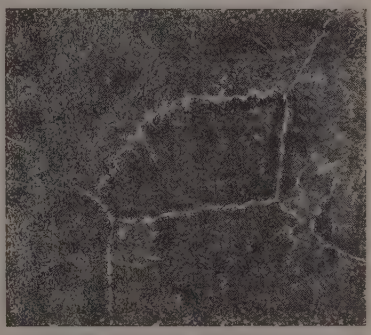
As the table shows, the general alloy yield is about 80—90 per cent with the exception of the alloying element № 7, where the total percentage yield goes down to 75 per cent. The low yield of alloys with a high Al content

(over 30 per cent) is due to the fact that a large part of the powdered aluminium in the charge oxidizes more strongly and quickly before it can get into the alloy. The percentage yield of chromium calculated by the quantity of chromium oxide taken also varies from 80 to 90 per cent, going down as far as 77 per cent in the alloying element № 7. The aluminium used for the reaction and its excess which goes into the formation of the chrome-aluminium alloy of the necessary composition, behaves in such a manner, that under normal reduction (dried reagents, absence of foreign admixtures, which aluminium could reduce, well pounded charge and the reagent flowing without spurts) its loss by burning is 5—10 per cent, as in the cases of the alloying element № 2 and 4. But in preparing alloying element № 1 the percentage yield of aluminium was only 55.37 per cent. Such a heavy loss of aluminium by burning is due to the charge not being pounded nor dried in this case, as a result of which the reaction itself was quite slow and sluggish.

The production of ternary alloy of iron-chrome-aluminium after the aluminothermic method, it should be noted, does not entail great difficulty either. Just as during the production of chrome-aluminium, the reaction goes on normally, giving a total percentage yield of the alloying element of 80—90 per cent.

In view of the comparatively high silica content in iron oxide there was up to 1 per cent of silicon in these alloys.

A distinctive feature in this case is that chromium oxide becomes reduced with greater difficulty in the presence of iron oxide, which tells on the percentage yield of chromium decreasing, giving instead almost a 100 per cent yield of iron.



×120. Etching with aqua regia.

The microstructure of these alloys was studied, while in cast conditions. The polished surfaces were prepared in the usual manner and were treated with aqua regia. We found it a distinctive feature that alloys which correspond to the region of solid solutions of aluminium in chromium [according to A. Bradley's data (4)] up to 17 per cent Al are not subject to etching with aqua regia and do not show any structure at all. Keeping the samples for 5—10 min. in aqua regia did not give any noticeable changes in the polished surface, which remained as shiny as it had been before being submerged for etching. Those regions where, according to the equlibrium diagram, one should expect the solid solution to decompose, i. e. the non-homogeneous regions, are easily subject to the action of aqua regia and the alloy shows a polyhedral structure in which a new phase may be distinguished.

A microphotograph of alloying element № 4 is given in the figure where we observe the formation of a new phase along the borders of and inside the grain, when the alloy is cooled in air.

Conclusions

1. By studying the aluminothermic process of producing alloys of chrome-aluminium we have shown that it is possible to prepare them.
2. The average yield of the alloying element in the laboratory is 80—90

per cent, the percentage chromium yield is also within these limits, and aluminium can be used up to 95 per cent.

3. Experiments on the production of ternary alloy of iron-chrome-aluminium also proved it possible to prepare these alloying elements after this method securing a yield of up to 80—85 per cent.

4. The microstructure of cast chrome-aluminium alloys in this region shows homogeneous solid solutions up to 17 per cent Al. If the Al percentage is higher, a new phase is precipitated from the solid solution in the solid state.

5. The possibility of obtaining alloys chrome-aluminium with quite satisfactory chromium and aluminium yield, the production of continuous homogeneous alloys which are free from other admixtures—gives grounds to start the production of these alloying elements on a commercial scale, which is necessary for preparing certain technical alloys, containing chromium and aluminium without carbon.

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CHIMIE

SUR LA COLORATION DES ARYLAMIDES DE L'ACIDE *p*-NITRO-PHÉNYLACÉTIQUE

(SYSTÈMES AUXO-ÉNOÏDES SÉPARÉS. VIII)

Par W. A. ISMAILSKI et E. A. SMIRNOV

(Présenté par A. E. Porai-Koschitz, de l'Académie, le 3.VII.1938)

Nous avons démontré dans nos communications précédentes (1) que les dérivés nitro-benzoylés des amines aromatiques contenant un groupe auxo complémentaire ont une coloration qui varie entre le jaune clair et le rouge foncé:

I. $O_2N-C_6H_4-CO\cdot NH-C_6H_4-(auxo)$.

Nous sommes arrivés à la conclusion que la structure imidole du groupe de liaison (—C(OH)=N—) et que, par conséquent, la présence de la double liaison entre C et N n'est pas indispensable pour l'apparition de la coloration dans les composés de ce genre.

L'article présent contient une description des dérivés de l'acide *p*-nitrophénylacétique (II, III, IV) où le groupe—CO.NH—est remplacé par le groupe—CH_2.CO.NH—:

II. $O_2N-C_6H_4-CH_2\cdot CO\cdot NH-C_6H_4-OCH_3$ presque incolore,

III. $O_2N-C_6H_4-CH_2\cdot CO\cdot NH-C_6H_4-OH$ presque incolore,

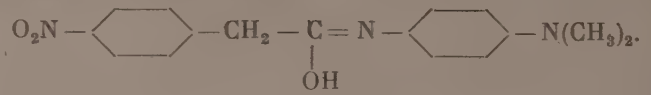
IV. $O_2N-C_6H_4-CH_2\cdot CO\cdot NH-C_6H_4-N(CH_3)_2$ orange.

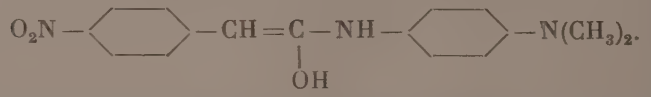
On y observe un affaiblissement considérable de la coloration en comparaison aux dérivés nitro-benzoylés (1) et nitro-benzylés (2) (v. tableau).

Cependant le dérivé diméthylaminé (IV) s'est déclaré orange. Au point de vue de la théorie de conjonction la formule IV ne peut servir d'explication à la coloration de ce composé (le système conjugué étant interrompu). Pour satisfaire aux conditions de cette théorie on est obligé de chercher cette explication dans la formation d'un système conjugué produit au moyen d'un réarrangement.

En admettant les conclusions de Mme Ramart-Lucas (3) concernant l'existence probable d'amides de l'acide acétylacétique (R.CO.CH_2.CO.NH.R)

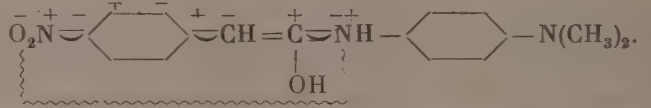
en trois formes—amide, imidole et énole—nous pouvons construire pour nos composés (outre le IV) des formules tautomères: la formule imidole (V) et la formule énole (VI):

V. 

VI. 

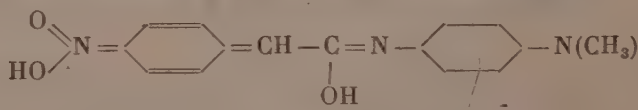
Cependant même dans les formes données il nous manque une chaine conjuguée ininterrompue entre les groupes NO_2 et $N(CH_3)_2$ (si nous n'admettons pas d'hypothèses complémentaires). Dans la formule V cette chaine est interrompue par le groupe—CH_2—et dans la formule VI—par le groupe—NH—.

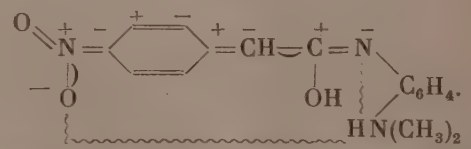
Mais on peut bien admettre que le groupe NH dans la formule VI soit l'auxo principal du chromosystème et que le groupe $N(CH_3)_2$ n'agit que d'une manière inductive (influence positivante). Nous pouvons alors construire le système conjugué endohalochrome (4) VII.

VII. 

Pourtant l'explication du phénomène de coloration par ce schéma est insuffisante car si le groupe NH y jouait le rôle de l'auxo principal [périchrome selon la terminologie de W. König (4)] une coloration visible devrait apparaître dans des composés ayant l'*auxo*=OH, OCH_3 et même dans ceux qui sont dépourvus du groupe auxo complémentaire (*auxo*=H), NH étant comme tel un auxo-groupe assez fort. Nous devrions observer les mêmes phénomènes que chez les dérivés nitro-benzylés, dont les composés les moins compliqués et dépourvus du second *auxo* (p-O_2N-C_6H_4—CH.NH—C_6H_5 et m-O_2N—C_6H_4—CH_2.NH—C_6H_5) ont une coloration intense (2). On est donc obligé d'admettre que la coloration est occasionnée non par le groupe NH, mais par le groupe $N(CH_3)_2$.

L'explication de la coloration par la théorie de conjonction ne peut donc se passer d'hypothèses complémentaires. Il paraît tout d'abord qu'une telle hypothèse permettant de construire une formule à chaine conjuguée continue entre NO_2 et $N(CH_3)_2$ se présente dans l'admission d'un s e c o n d réarrangement quinoïde se produisant simultanément au regroupement imidole (VIII) avec formation d'un système de conjonction (IX):

VIII. 

IX. 

Cette interprétation est cependant peu probable car l'étude de la provenance de la coloration des nitro-benzyl-arylamines par W. A. Ismailski

et V. I. Stavrovskaia (2) nous amène à la conclusion que l'hypothèse du regroupement quinoïde en une acinitro-forme, suivi d'une formation d'un conjoncteur d'ammonium, par la migration du proton, ne suffit pas à expliquer le phénomène de la coloration de ces composés (contenant le même groupement $O_2N{-}C_6H_4{-}CH_2$).

Les phénomènes suivants doivent être considérés comme de forts arguments contre la théorie de l'acinitro-structure quinoïde des dérivés nitro-benzylés:

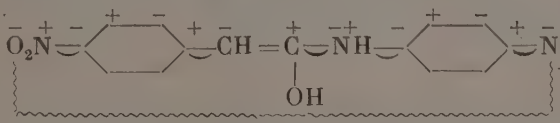
1) la présence d'une coloration du même ordre lorsque NO_2 se trouve en *m*-position,

2) l'effet hypsochrome en cas d'un remplacement du groupe arylamino par celui d'alkylamino ou amino et,

3) la présence de propriétés basiques très fortes chez ces derniers composés, quoique les conditions pour le regroupement et la neutralisation du groupe acinitro y sont plus favorables.

Une autre hypothèse complémentaire permettant de construire un système de conjonction avec participation du groupe $N(CH_3)_2$ est fondée sur l'admission que l'atome nitrogène dans le groupe NH fonctionne comme le dipole $\overset{-}{N}\overset{+}{}$, jouant le rôle de conducteur d'influences. Cette admission est faite par W. König pour expliquer l'influence bathochrome du second groupe auxochrome dans une série de cas [W. König, l. c. (4), formules XX, etc.]. Nous obtenons alors la formule X:

X.



Ce schéma parait donner une explication tout à fait satisfaisante de l'influence du groupe $N(CH_3)_2$ sur l'apparition de la coloration chez un dérivé nitro-phénylacétique donné. Cependant quelques objections s'y opposent: a) le fait qu'un composé à chaîne conjuguée aussi longue possède une coloration moins profonde que le dérivé nitro-benzoylé correspondant contenant une chaîne conjuguée plus courte et b) l'absence de coloration chez les composés contenant l'auxo-groupe OCH_3 ou OH.

Dans ce dernier cas en présence d'une structure énolique une formation de systèmes de conjonction plus courts sous participation d'un auxo-groupe —NH—fort, comme la formule VII l'indique, suffirait pour occasionner l'apparition de la coloration (son apparition sous participation d'hydroxyle énole, qui est un auxochrome plus faible, est moins probable).

Notre étude donne lieu aux conclusions suivantes:

1. Les arylamides de l'acide *p*-nitro-phénylacétique sont beaucoup plus aptes aux regroupements tautomères que les dérivés nitro-benzoylés correspondants. Le fait que la coloration de ces composés est tout de même moins prononcée, que celle des dérivés nitro-benzoylés, indique que les phénomènes de la coloration sont conditionnés par une structure déterminée, les atomes du système se trouvant en un état dynamique spécial (chromo-état), et que l'augmentation du nombre d'oscillations tautomères possibles n'est point un facteur favorable à l'approfondissement de la couleur.

2. La constitution des arylamides de l'acide *p*-nitro-phénylacétique permet de construire des systèmes de conjonction différents. Mais l'explication de la coloration par ces schémas ne peut être considérée comme strictement prouvée et n'explique pas l'effet hypsochrome, en comparaison aux dérivés nitro-benzoylés, produit par l'introduction du groupe CH_2 entre $O_2N{-}C_6H_4$ et—CO—. Les schémas les moins probables à notre

avis sont ceux qui comprennent le réarrangement quinoïde du groupe $O_2N—C_6H_4—CH_2—$ dans une acinitro-forme.

3. Vu que les phénomènes de la coloration, comme nos études l'ont démontré, peuvent être observés même dans des cas où les regroupements tautomères et la formation de la chaîne conjuguée entre NO_2 et auxo sont impossibles, nous sommes tentés de croire que dans ce cas aussi c'est l' a c t i o n m u t u e l l e d e s d e u x s y s t è m e s n i t r o - é n o ï d e e t a u x o - é n o ï d e séparés, qui joue le rôle de facteur principal déterminant les phénomènes de la coloration des dérivés de l'acide *p*-nitro-phénylacétique. Ce qui renforce encore cette conclusion c'est qu'elle offre une explication aisée de l'effet hypsochrome produit par l'introduction du groupe CH_2 dans les nitro-benzoyle-arylamides entre $O_2N—C_6H_4$ et —CO— (XI—XII), ainsi que l'effet hypsochrome du à l'introduction du groupe —CO—dans les nitro-benzyle-arylamines entre les groupes—CH_2—et—NH— (XIII—XII) (v. tableau).

Tableau

Coloration des composés du genre $O_2N—C_6H_4—Q—C_6H_4—$(*auxo*)

auxo	*Q*=—CO.NH—	*Q*=—CH_2.NH—	*Q*=—CH_2.CO.NH—
OCH_3 . .	Jaune	Jaune } deux Orange } formes	Presque incolore
OH . . .	Orange	Rouge-brun	» »
$N(CH_3)_2$.	Rouge profond	Rouge foncé	Orange

L'explication de ces phénomènes peut être trouvée à base d'une analyse des changements dans les systèmes initials nitro-énoïde (B) *et auxo-énoïde* (A) *particuliers à la suite de l'introduction des groupes mentionnés.*

XI. $O_2N—C_6H_4—CO\cdot NH—C_6H_4—$(*auxo*). (B, A)

XII. $O_2N—C_6H_4—CH_2\cdot CO\cdot NH—C_6H_4—$(*auxo*). (B′, A)

XIII. $O_2N—C_6H_4—CH_2\cdot NH—C_6H_4—$(*auxo*). (B″, A′)

Les composés XI et XII contiennent le même système auxo-énoïde (A) et de différents systèmes nitro-énoïdes (B et B′). Le système nitro-énoïde B′ est affaibli en comparaison au système B par la séparation du groupe CO du système des double liaisons du noyau auquel le groupe NO_2 est rattaché. Si les influences contra-polarisantes exercées par le groupe CO sur NO_2[1]), qui renforcent les propriétés chromophores du système nitro-énoïde, peuvent être transmises par le groupe CH_2, ce n'est que dans un degré beaucoup plus restreint, que dans des composés nitro-benzoylés où ce groupe est directement lié au noyau.

D'autre part, l'effet hypsochrome du groupe CH_3 introduit dans la partie nitro-énoïde de la molécule peut être comparé à l'effet hypsochrome

observé à la suite de l'introduction du groupe CH_3 dans le composant nitroïde des combinaisons moléculaires (5).

La comparaison des composés XII et XIII montre qu'ils contiennent des systèmes nitro-énoïdes B′ et B″ semblables ($O_2N—C_6H_4—CH_2$—dans les deux cas) et des systèmes auxo-énoïdes A et A′ différents. Dans le système A chez les dérivés de l'acide nitro-phénylacétique la fonction auxochrome du groupe NH est très affaiblie par l'influence du groupe CO (groupe acyl-amino), en comparaison aux dérivés nitro-benzylés du système A′ (groupe alkyl-amino). Le système di-auxo A est en conséquence beacoup plus faible en comparaison au di-auxo-système A′. D'autre part, l'introduction du groupe négativant CO ne renforce le système nitro-énoïde que médiocrement, car ce groupe est séparé des liaisons doubles du noyau et du NO_2 par le groupe méthylenique, étant au surplus considérablement affaibli par le groupe voisin NH. Il en résulte un grand affaiblissement de coloration des dérivés de l'acide nitro-phénylacetique (XII) en comparaison aux dérivés nitro-benzylés (XIII).

Partie expérimentale

I. *4-[4′-nitro-phénacétamino]-anisol* (II) (*p*-anisidide de l'acide *p*-nitro-phénylacétique).

Ce produit a été obtenu d'une manière analogue à celle employée dans les cas des dérivés nitro-benzoylés (1), c'est-à-dire à l'aide de l'action du chloranhydride sur l'amine en présence du bicarbonate de soude. Deux récristallisations dans l'alcool, une dans l'acétone. Substance presque incolore en menus cristaux. Point de fusion 189°.

Analyse. Subst. 0.1576 g; N_2=13.23 cm³ (16°, 755 mm). Calculé pour $C_{15}H_{14}O_4N_2$: N 9.79%. Trouvé: N 9.76%.

II. *4-[4′-nitro-phénacétamino]-phénol* (III) (*p*-oxy-anilide de l'acide *p*-nitro-phénylacétique).

Deux récristallisations dans l'acétone, une dans l'alcool. Substance très faiblement colorée (presque incolore). Point de fusion 233°. Assez soluble dans l'alcool et l'acétone, insoluble dans le benzène et l'éther.

Analyse. Subst. 0.1523 g; N_2=13.46 cm³ (15°, 756 mm). Calculé pour $C_{14}H_{12}O_4N_2$: N 10 29%. Trouvé: N 10.36%.

III. *4-[4′-nitro-phénacétamino]-diméthylaniline* (IV) (*p*-diméthylamino-anilide de l'acide *p*-nitro-phénylacétique).

Deux récristallisations dans l'acétone, une dans l'alcool. Cristaux rouges-orangés. Point de fusion 217°. Soluble dans l'acétone et l'alcool (moins aisée cependant que celle du dérivé OH correspondant), insoluble dans le benzène et l'éther.

Analyse. Subst. 0.1362 g; N_2=15.85 cm³ (18°, 752 mm). Calculé pour $C_{16}H_{17}O_3N_3$: N 13.33%. Trouvé: N 13.42%.

Manuscrit reçu
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LITTÉRATURE CITÉE

[1] В. А. Измаильский и Е. А. Смирнов, Ж.О.Х., VII, 513, 523 (1937); Bull. Soc. Chim. (5), 4, 81, 94 (1937); C. R. Acad. Sci. URSS, XIV, № 1, 17 (1937); Ж.О.Х. (1938). [2] В. А. Измаильский и В. И. Ставровская, Ж.О.Х., VIII (1938). [3] Ramart-Lucas, Naik et Trivedi, Bull. Soc. Chim., (5), 1, 4 (1934). [4] W. König, Journ. prakt. Chem., (2), 112, 14 (1926). [5] P. Pfeiffer, Org. Molekularverbindungen, 228 (1922).

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CHIMIE

SUR LA COLORATION DES ARYLAMIDES DE L'ACIDE *p*-NITRO-HYDROCINNAMIQUE

(SYSTÈMES AUXO-ÉNOÏDES SÉPARÉS. IX)

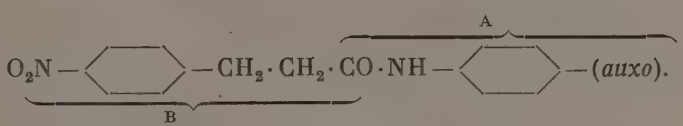
Par W. A. ISMAILSKI et E. A. SMIRNOV

(Présenté par A. E. Porai-Koschitz, de l'Académie, le 3. VII. 1938)

Au cours de nos recherches sur le problème de la provenance de la coloration dans les composés du genre I nous avons fait la synthèse de la série

I. $$O_2N-C_6H_4-Q-C_6H_4-(auxo)$$

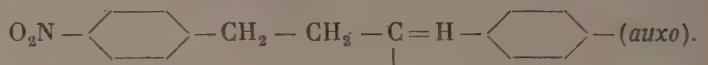
de dérivés de l'acide nitro-hydrocinnamique où $Q=-CH_2.CH_2.CO.NH-$. Cette série diffère des nitro-benzoyl-arylamides par la présence de deux groupes méthyléniques entre le noyau benzénique et le groupe CO (II):

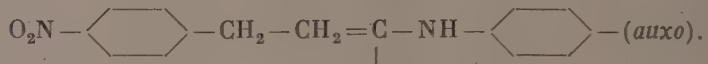
II. 

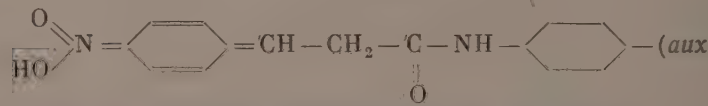
La synthèse de ces composés offre un intérêt spécial car elle permet de vérifier les conclusions de nos communications précédentes ([1], [2], [3]): 1) la coloration des nitro-benzoyl-arylamides ($Q=-CO\cdot NH-$) est occasionnée par l'action mutuelle de deux systèmes de la molécule nitro-énoïde et auxo-énoïde séparés, exercée directement selon le genre de l'action mutuelle entre les nitrocorps et les amines et phénols dans les composés complexes et 2) l'apparition de la coloration visible dans les composés du genre I est possible même lorsque le groupe de liaison Q ne contient pas de double liaison conjugant les deux noyaux, c'est-à-dire quand l'auxo-groupe et le nitro-groupe ne forment pas les groupes extrêmes d'une chaîne conjuguée commune.

La formule II contient deux systèmes isolés A et B. Contrairement à ce que nous observons dans les composés contenant le groupe —CO.NH—, la formation d'une chaîne conjuguée ininterrompue est impossible dans ce cas même au moyen d'un regroupement tautomère en forme imidole (III), énole (IV) ou quinoïde (V). Au point de vue de la théorie des liaisons conjuguées la coloration dans ce cas devrait être absente. Mais du point de vue de notre admission de la possibilité d'une action mutuelle de deux systèmes

séparés lors de l'introduction d'un auxo-groupe fort on pourrait s'attendre à l'apparition de la coloration:

III. 

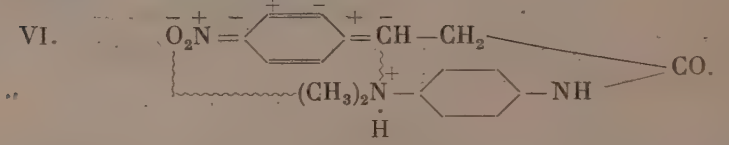
IV. 

V. 

Dans les composées contenant un *auxo*=OCH_3 ou OH (formule II), dont nous avions fait la synthèse, la coloration était sensiblement affaiblie en comparaison aux nitro-benzoyl-arylamides respectifs [voir tableau de la communication précédente ([3])]: le composé contenant OCH_3 est presque incolore, le composé contenant OH—jaune clair.

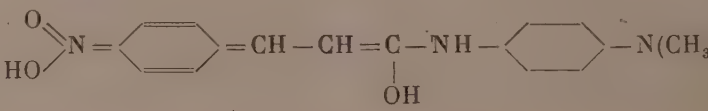
Cependant le composé contenant un *auxo*=$N(CH_3)_2$ s'est manifesté rouge-orangé confirmant ainsi notre attente. Ce phénomène ne peut être expliqué à base de la théorie de conjonctions, même au moyen des formules III, IV et V. Des hypothèses complémentaires sont nécessaires.

On pourrait supposer que cette coloration est causée par la présence d'une structure quinoïde dont les propriétés chromophores sont renforcées par l'action d'un groupe de cations d'ammonium formé par l'adjonction du proton au groupe $N(CH_3)_2$ (qui selon W. König joue le rôle de conjoncteur—«Konjugens») VI ou, si l'on conçoit le phénomène dans le style de l'hypothèse de M. Dilthey et Wizinger, par la formation d'un atome C ionoïde.

VI. 

Mais cette explication est de même peu probable en considération de ce qui était déjà dit dans notre communication précédente et dans celle de W. A. Ismailski et W. I. Stavrovskaïa ([4]) sur la coloration des nitro-benzyl-arylamines. Nous pouvons nous attendre à ce que dans les combinaisons de type II un effet bathochrome va apparaître si NO_2 est introduit dans la *meta*-position, c'est-à-dire lorsqu'un regroupement quinoïde est impossible.

D'autre part, si les deux réarrangements tautomères—quinoïde et énole—accompagnés de la formation de la structure VII se produisent simultanément, la formation d'une chaîne conjuguée ininterrompue devient tout de même possible:

VII. 

Il est possible de construire pour la structure VII toute une série de systèmes conjugués fermés par conjonction. Mais il est difficile de trouver une base à cette formation et d'expliquer à leur aide l'ordre de la coloration observée.

L'hypothèse de l'apparition d'un système de liaisons conjuguées, par exemple, avec la participation de $N(CH_3)_2$ comme groupe qui forme un conjoncteur cationique, d'une manière analogue à la formule VI, au moyen de l'addition d'un proton, ainsi que l'hypothèse de la structure quinoïde doivent être reconnues insuffisantes en considération de ce qui était dit à propos des nitro-benzyl-amines ([4], [3]).

Le réarrangement énolique lui-même est peu probable, car le groupe CO doit être en ce cas assez clos à la suite de l'action mutuelle avec le groupe NH [dont les propriétés basiques sont renforcées par l'influence positivante du groupe $N(CH_3)_2$], et il est peu probable qu'il exerce une influence assez réactivante sur le groupe voisin CH_2. Autrement dit, si nous admettons dans ce cas la possibilité d'une tautomérie, c'est la forme imidole—C(OH)=N—, ayant dans la molécule un groupe CH_2, interrompant le système conjugué, qui est la plus probable.

Nous arrivons donc cette fois aussi à la conclusion que ce n'est pas parmi les phénomènes mentionnés de regroupement, accompagné de la formation dans le groupe de liaison de doubles liaisons conjuguées, qu'il faut chercher la cause de la coloration des composés du genre II. Conformément à notre point de vue exposé dans nos travaux précédents nous croyons que c'est l'action mutuelle des systèmes nitro-énoïde (B) et auxo-énoïde (A) séparés qui est le facteur principal déterminant les phénomènes de la coloration chez les composés de ce genre (II).

Si la méthode d'analyser l'état de la coloration, décrite dans nos travaux précédents [pour les nitro-benzoyl-arylamides ([1]) et les amides de l'acide nitro-phénylacétique ([3])], est employée du point de vue de la structure des systèmes particuliers nitro-énoïdes et auxo-énoïdes, l'effet hypsochrome observé lors de l'introduction dans la molécule du nitro-benzoyl-arylamide entre $O_2N—C_6H_4—$ et—CO—du groupe $—CH_2 \cdot CH_2—$reçoit une explication tout à fait satisfaisante: le système chromophore assez fort $O_2N—C_6H_4—CO—$se trouve affaibli à la suite d'un détachement du groupe CO du système $O_2N—C_6H_4—$ et de la disparition ou de l'anéantissement presque total de son influence contra-polarisante ([1]).

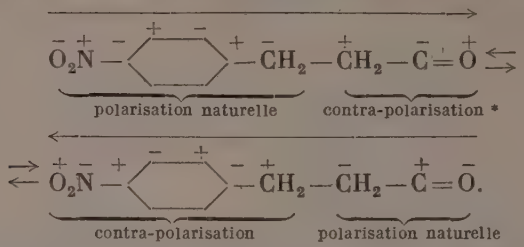
Il est plus difficile d'expliquer la coloration plus profonde des dérivés de l'acide *p*-nitro-hydrocinnamique que celle des dérivés correspondants de l'acide *p*-nitro-phénylacétique, c'est-à-dire d'expliquer pourquoi le système $p\text{-}O_2N—C_6H_4—CH_2—CH_2—CO—$est plus chromophore que le système $p\text{-}O_2N—C_6H_4—CH_2—CO—$.

Ce fait pourrait bien s'expliquer en premier lieu par l'alourdissement du groupe alcylique, c'est-à-dire par une influence plus forte du groupe $—CH_2 \cdot CH_2—$en comparaison au groupe$—CH_2—$sur les systèmes chromophores voisins, ce qui est conforme aux observations de plusieurs savants ([5]) qui constatent un déplacement bathochrome de la bande d'absorption des cétones aliphatiques à cause d'un allongement de la chaîne carbonique des alcyles.

D'autre part il nous semble qu'il serait intéressant de vérifier expérimentalement la question suivante: le phénomène mentionné ne serait-il pas le résultat d'influences polarisantes contraires (contra-influence), transmises par le système$—CH_2—CH_2—$; dans le cas donné l'influence serait

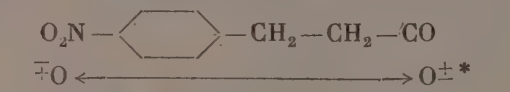
transmise comme résultat d'une oscillation résonnante des deux parts du système donné se trouvant dans de différentes stades de polarisation:

VIII.

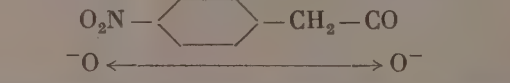


Dans le cas de position paire du CO par rapport à NO_2 (IX) il y a la possibilité d'influences contra-polarisantes renforçant les propriétés chromophores du système donné en comparaison au système X, qui ne possède que la possibilité d'influences polarisantes coïncidantes**:

IX.



X.



Si l'alternation des influences polarisantes par voie d'une chaîne saturée des atomes de carbone est en général peu probable, la discussion du problème de la possibilité d'influences contraires ne peut pas être évitée sans un fondement expérimental dans le cas donné où l'influence est exercée des deux côtés sur un système ne contenant que deux C-atomes.

Partie expérimentale

Synthèse de l'acide p-nitro-hydrocinnamique. La synthèse de l'acide cinnamique a été faite selon Kalnin (7) [substitution de l'acétone de sodium (8) par le potasse, échauffement pendant deux heures]. Réduction par l'amalgame du sodium. Point de fusion de l'acide hydrocinnamique 48°.

La nitration de l'acide hydrocinnamique a été faite à l'aide du nitrate d'ammonium. L'acide hydrocinnamique (0.1 mol.) a été ajouté par petites doses à la dilution du nitrate d'ammonium (0.8 mol.) dans de l'acide sulfurique à poids spécifique 1.84 (1 mol.) à 5—10°. Après une demi-heure de repos à 5° la solution a été versée dans l'eau, le précipité filtré et lavé à l'eau.

Pour éloigner l'*o*-isomère plus soluble dans l'eau chaude le produit a été bouilli deux fois avec une petite quantité d'eau et filtré et lavé chaque fois avec de l'eau chaude. Après une récristallisation dans l'eau des cristaux en forme d'aiguilles ont été obtenu. Point de fusion 163—164°. [Conformément aux données de Glaser et Buchanan (9)].

4-[4'-nitro-hydrocinnamoyl-amino]-anisol (I, *auxo*=OCH_3) *p*-anisidide de l'acide *p*-nitro-hydrocinnamique). Ce produit a été obtenu de *p*-anisidine et chlorure du *p*-nitro-hydrocinnamoyl par le même procédé à l'aide duquel nous avions obtenu les nitro-benzoylarylamides (1).

Une récristallisation dans l'acétone, une dans l'alcool méthylique, une dans l'alcool éthylique. Substance cristallique presque incolore. Point de fusion 183°. Soluble dans l'acétone et dans l'alcool méthylique et éthylique; insoluble dans le benzène et l'éther.

Analyse. Subst. 1597 g; N_2=12.98 cm^3 (17°, 754 mm). Calculé pour $C_{16}H_{16}O_4N_2$: N 9.33%. Trouvé: N 9.42%.

4-[4'-nitro-hydrocinnamoyl-amino]-phénol (II, *auxo*=OH) (*p*-oxy-anilide de l'acide *p*-nitro-hydrocinnamique). Récristallisation triple dans l'alcool méthylique avec addi-

* Les signes ± dans les schèmes des systèmes contra-polarisés indiquent la direction du déplacement de la polarisation. Le signe + sur O, par exemple, indique la diminution de la charge négative.

** À propos de la méthode de formulation des influences polarisantes contraires voir W. A. Ismailski et A. M. Simonov (6).

tion de l'eau. Point de fusion 181°. Cristaux jaune clair. Soluble dans l'alcool et l'acétone; insoluble dans le benzène et l'éther.

Analyse. Subst. 1594 g; N_2=13.53 cm³ (17.5°, 768 mm). Calculé pour $C_{15}H_{14}O_4N_2$: N 9.79%. Trouvé: N 9.87%.

4-[4-nitro-hydrocinnamoyl-amino]-diméthyl-aniline (II, *auxo*=N(CH_3) (*p*-diméthyl-amino-anilide de l'acide *p*-nitro-hydrocinnamique).

Une récristallisation dans la pyridine, une dans l'acétone, une dans l'alcool. Cristaux rouge-orangés. Point de fusion 225.5°. Très soluble dans la pyridine, peu soluble dans l'alcool et l'acétone, insoluble dans le benzène et l'éther.

Comme le dérivé *p*-nitro-cinnamilique correspondant

XI. p'—O_2N—C_6H_4—CH=CH—CO—NH— C_6H_4—N(CH_3)$_2$—p

se trouvant en forme de cristaux a une coloration rouge très profonde, presque noire (tandis qu'elle est bordeau quand ce produit est en forme de poudre), la coloration du dérivé *p*-nitro-hydrocinnamoylique obtenu par nous pouvait etre due à la présence dans notre produit d'une petite quantité d'un dérivé pas complétement hydrogéné*.

C'est pour ça que nous avions cru nécessaire de vérifier nos conclusions par la synthèse de ce composé au moyen d'un autre procédé, à savoir—en faisant cette synthèse de l'acide hydrocynnamique obtenu de l'éther malonique et de la chlorure benzylique. Le produit ainsi obtenu avait la même coloration rouge-orangée et le même point de fusion 225.5°:

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LITTÉRATURE CITÉE

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* Comparer avec les données de Ramart-Lucas ([10]) concernant l'influence de l'admixtion du stilbène sur le spectre d'absorption du dibenzyle.

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TECHNICAL CHEMISTRY

SOLUTION OF COALS

By M. K. DJAKOVA, A. V. LOZOVOJ, T. G. STEPANZEVA and S. A. SENJAVIN

(Communicated by I. M. Gubkin, Member of the Academy, 26.VII.1938)

This investigation was undertaken in order to devise methods of simplifying the technically complicated and difficult procedure of hydrogenating solid fuel into motor fuel.

We find that the simplest way of coal liquefaction is by hydrogenating organic coal masses in the form of an ash-free colloidal coal solution. This would reduce the complicated procedure of hydrogenating coal down to the comparatively simple hydrogenation of liquid fuels. Commercially obtainable, cheap liquid products such as tars, mazouts etc. should be used as solvents for the coal. Coal and oil shale solutions in anthracene oil, primary tars and in other organic solvents may be used up not only as intermediate products for hydrogenation into motor fuel, but possibly as a heavy liquid fuel as well.

The literature on dissolving coal in commercial solutions at high temperatures is rather scanty. This problem was studied by A. Pott and H. Broche(1) who dissolved a number of German coals in mixtures of phenols with tetralene and naphthalene, by a Spanish chemist, J. M. Pertierra (2), who showed that it was possible to liquefy 87—95 per cent of organic coal mass in anthracene oil, primary tars and in mixtures of tetralene and phenol, by heating to 410° under a pressure of 213—240 atm. of hydrogen. A. Gillet (3) dissolved coals in anthracene oil and β-naphthol at atmospheric pressure. Blumener's process (4) which seems of late to win a place in industry is a combination of the methods of suspending and dissolving coals and that of cracking coal solutions and carbonizing coals. M. I. Kusnezov and L. L. Nesterenko (5) have recently reported the results of their experiments on the solution of some Soviet coals in anthracene, naphthalene, pyridine, phenol. This is nearly all the literature we dispose of on the solution of coals.

In this preliminary paper we give the results of experiments made to find the action of some commercial solvents on a number of typical representatives of solid fuel. We do not intend giving accurate accounts of the method of the experiments, nor of the objects used in the investigation, leaving this off for some future work.

Raw materials. The following were used as solvents: 1) the primary tar fraction of humic-sapropelite coals; 2) anthracene oil; 3) shale oil distillate.

The following were used as solutes: 1) humic-sapropelite coal from Eastern Siberia (ash 12.7%); 2) old humic brown coal (ash 25.54%); 3) young brown

coal (ash 20.06%); 4) sapropelite coal (ash 23.2%); 5) oil shale (ash 39.44%). Before dissolving, they were pulverized to a gristfineness of 100 mesh and dried.

Method. The coals and oil shale were dissolved in a half-liter rotating autoclave of the «Bergius» type. 200—300 g of a mixture of the coal and the solvent were put into the autoclave. It took 25—30 min. to heat the autoclave up to the initial temperature (275°), then the temperature was slowly raised for several hours to 375—410°. The pressure in the autoclave during the process of solution was generally 20—80 atm., the residual pressure, 5—15 atm.

In order to increase the solubility of the coal most of the experiments were arranged in several stages: after heating the mixture at the chosen temperature the contents of autoclave were filtered and the coal solution was separated from the undissolved coal residue; the latter was again put into the autoclave together with a fresh portion of solvent and dissolved at a higher temperature than in the preceding experiment. Such a solution of the residual coal was repeated several times.

The degree to which the organic coal mass had dissolved was determined by the increase in the ash residue. The residue was for this purpose washed with the solvent, and then with benzene, until it became completely colourless, then it was dried at 100° to constant weight and ash-content determined in an average sample. The per cent of organic coal mass dissolved (x) was calculated according to the formula:

$$x = 100\left[1 - \frac{a\,(100 - b)}{b\,(100 - a)}\right],$$

where a is the ash of the initial coal in per cent, b is the ash of the coal residue after dissolving in per cent.

Results of the Experiments on the Solution of Coals and Oil Shale. The experiments were conducted in order to dissolve the greatest possible amount of organic coal mass, to determine the fitness of some typical kinds of solid fuel to be worked over after this method, and to find the optimal temperature, time and concentration of the initial oil mixture. Besides this the influence of hydrogen in small concentration and that of the humidity of the coal on the results of solution were studied. The most interesting data from these experiments are given in Tables 1 and 2. From these data together with other material which we did not include in the tables for lack of space, we draw the following conclusions:

1) The following dissolve the most easily and completely in tars and anthracene oil: sapropelite coal (80—86 per cent of the organic mass) and shale (80 per cent of the combustible mass). Young humic brown coal also dissolves easily (70—75 per cent of organic mass). Old brown coals dissolve with greater difficulty in anthracene oil and tars, but yet quite effectively (56—60 per cent). There are no grounds at present to affirm that the degree of solution attained by us for the above substances is the limit one.

2) The typical aromatic solvent—anthracene oil—dissolves the organic mass of humic and humic-sapropelite formations better than primary tars.

3) At temperatures under 325° coals and oil shale dissolve very little. At temperatures above 410° they stop dissolving and often become coked. Each kind of coal apparently has one or several comparatively narrow temperature ranges which are favourable for its solution, and also its upper temperature limit.

4) The tables show the highest figures as to the duration of the experiments and the number of stages of solution. The results of our latest experi-

Table 1

Solution of Brown Coals

№ experiment	Raw materials: Name of solute and quantity in g	Raw materials: Name of solvent and quantity in g	Heating conditions: Duration in min.	Heating conditions: Temperature range in °C	Ashes of the residue in per cent	Percentage of coal that passed into solution (calculated by the ash of the residue), counting only combustible mass	Remarks
106	Humic-sapropelite coal from Eastern Siberia, 90	Anthracene oil, 210	120	275—350	17.24	30.2	
109	Residue from experiment № 106, 66.8	» » 156.7	90	350—380	20.2	42.5	
111	Residue from experiment № 109, 50.5	» » 117.8	90	380—400	24.9	56.1	
110	Humic-sapropelite coal from Eastern Siberia, 90	Anthracene oil, 210	120	275—350	17.7	32.3	Experiments were conducted in hydrogen. Initial pressure 10 atm.
112	Residue from experiment № 110, 73.4	» » 171.2	90	350—380	22.95	51.2	
114	Residue from experiment № 112, 51.5	» » 122	90	380—400	28.0	62.6	
97	Old humic brown coal, 60	Anthracene oil, 140	180	275—325	26.72	6.0	
100	Residue from experiment № 97, 50	« » 166.5	90	325—350	33.6	32.2	
103	Residue from exp. № 100, 42.3	» » 90	90	350—380	36.5	40.3	
104	Residue from exp. № 103, 38.0	» » 90	90	380—400	38.8	45.9	
107	Residue from exp. № 104, 27.5	» » 84.2	90	400—410	44.7	57.6	
3	Young brown coal, 90	Anthracene oil, 210	100	275—380	47.14	71.8	
5	Residue from exp. № 3, 27	» » 87	90	380—410	50.32	75.4	
32	Young brown coal, 60	Primary tar, 240	180	275—380	42.45	69.8	Ash content of initial coal— 18.2 %

Table 2

Solution of Sapropelite and Oil Shale

№ experiment	Raw materials		Heating conditions		Ashes of the residue in per cent	Percentage of coal that has passed into solution (calculated by the residue), counting only combustible mass
	Name of solute and quantity in g	Name of solvent and quantity in g	Duration in min.	Temperature range in °C		
125	Sapropelite, 90 . .	Anthracene oil, 210	150	275—375	27.6	20.8
132	Residue from exp. № 125, 73 . . .	» » 170.3	60	375—400	68.5	86.1
105	Sapropelite, 90 . .	Shale oil, 210	150	275—375	37.2	48.9
121	Residue from exp. № 105, 41 . . .	» » 96	90	375—390	60.8	80.5
123	Sapropelite, 60 . .	Primary tar, 140	240	275—400	59.7	79.7
80	Shale, 60	Shale oil, 140	60	275—325	—	—
86	Residue from. exp. № 80, 31.8 . .	» » 68.2	90	325—350	—	—
92	Residue from. exp. № 86, 26 . . .	» » 75	90	350—380	75.2	78.5
95	Residue from. exp. № 92, 23 . . .	» » 77	90	380—400	76.64	80.1

ments suggest it to be possible to greatly decrease the duration of heating and the number of times the solvent is changed.

5) Increasing the proportion of coal in the initial coal and oil mixture from 20 to 50 per cent (tried by us in the case of young brown coal) lowers the degree of solutions of the organic mass comparatively little, greatly increasing the coal concentration in the coal solutions produced (from 12.1 to 29.7 per cent). The high per cent of coal load makes it difficult to filter off the coal solution from the undissolved residue.

6) If the coal is dried to 1—1½ per cent humidity, it dissolves a little better than humid coal.

7) Dissolving the coal in an atmosphere of hydrogen under slight pressure gives better results.

The per cent of organic mass of the coal in the solutions produced fluctuated from 12 to 30 per cent. By distilling the coal solution it is possible to raise the coal concentration in the solution to 45—50 per cent. Such a solution melts at 35—45°, is quite homogeneous and slightly mobile at 50—60°. Orientation experiments showed that these solutions are excellent material for hydrogenation into motor and Diesel fuel. Under such treatment the pitch-like coal solution easily turns into liquid products.

The results of this investigation made us confident that this new method of chemical solid fuel technology has great prospects.

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TECHNICAL CHEMISTRY

ON EGYPTIAN BLUE AND REFRACTORIES FOR COPPER SMELTING FURNACES

By B. V. IVANOV, A. I. ZVETKOV and I. M. SHUMILO

(Communicated by F. Loewinson-Lessing, Member of the Ac demy, 27.VI.1938)

In the course of petrographic studies of the refractories in non-ferrous metallurgy a beautiful blue mineral has been discovered in Dinas brick of the smelting furnace roof of the Kyshtym plant; a detailed investigation showed this mineral to be Egyptian blue. Such a formation of Egyptian blue in the technical products of the silicate industry has been recorded neither in Russian nor in foreign literature; yet this mineral possesses certain quite peculiar properties and the history of its practical use is of the greatest interest.

The Dinas brick in which Egyptian blue has been discovered is characterized by a distinct zonality. The following three zones may be established in the interval between the cold and the warm edge.

1. The unaltered zone of a light-yellow colour consists of fragments of quartzite and quartz partly replaced along the fractures and sometimes completely replaced by metastable christobalite. The cement contains an abundance of pseudowollastonite (α-$CaSiO_3$) and a smaller quantity of lanceolate tridymite twins.

2. The transitional zone, green with blue spots up to 15 mm thick. Here the character of transformation of the clastic material is similar to that of the unaltered zone but somewhat more intensive; the composition of the cement is essentially different. Along with the unaltered zone are present in the cement: wollastonite (β-$CaSiO_3$), a greenish-brown silicate of the vogtite type, a black opaque substance (CuO) and tridymite; further these are accompanied by the above-mentioned transparent blue mineral. This is closely associated with copper oxide and pistachio-green glass, forming attachments of bizarre shape.

3. The tridymite zone, brownish-red, compact, up to 115 mm thick. Towards the hot edge of the brick the quantity of the clastic quartzite material diminishes as it is gradually replaced by an opaque isothropic substance. The enrichment of the cement in tridymite proceeds in the same direction. Aside from tridymite the following constituents are present in the cement: a reddish-brown opaque substance (Cu_2O), small quantities of iron oxides, and in the areas lying close to the flame—metallic copper.

Thus, the blue mineral is confined exclusively to the transitional zone, to the part of the brick subjected to the influence of comparatively low temperatures. This mineral has been isolated and subjected to the most complete crystallographic, optical and chemical investigation.

The tetragonal symmetry of the mineral and the combination of $a:b:c=1:1:0.74447$, comparatively few simple forms, have been measured by means of a goniometer.

The forms $\{110\}, \{100\}, \{111\}, \{101\}$ and $\{001\}$ have been combined. The crystals show a tendency to produce lamellar forms along the basopinacoid.

The light refraction measured after the immersion method is as follows:

$$\left.\begin{aligned} N_0 &= 1.635 + 0.001 \\ N_e &= 1.604 \end{aligned}\right\} \quad N_0 - N_e = 0.031$$

Optical sign—negative. Pleochroism: X=light-pink, Z=dark-blue. Cleavage along the basopinacoid.

The material used for chemical analysis has been thoroughly purified, but owing to an extremely fine and intimate adherence of the blue mineral to the other constituents of the brick, it has been impossible to bring about a thorough purification. Therefore the data of the analysis show only the qualitative composition of this interesting mineral, but do not permit of establishing its formula.

Below are given the analyses of minerals and bricks according to zones.

Oxides	Blue mineral	Transitional zone	Tridymite zone
SiO_2	69.18	88.84	79.62
TiO_2	0.11	0.02	—
Al_2O_3	0.78	0.76	0.30
Fe_2O_3	0.22	0.28	None
FeO	—	0.72	0.19
MnO	—	0.01	0.01
CaO	13.04	2.82	0.13
MgO	—	0.01	None
CuO	16.32	7.09	None
Cu_2O	—	None	19.32
Cu	—	None	0.48
Total	99.65	100.55	100.05

A comparison of the results shows that the mineral under investigation is doubtlessly a compound of copper oxide.

The bulk of all the data, except goniometric measurements carried out for the first time, makes it possible to identify this mineral as a compound interesting in many respects, i. e. with Egyptian blue—a double silicate of copper oxide and calcium, for which Fougué (1) has suggested the formula $CuO.\ CaO.\ 4\ SiO_2$. This formula has been disputed by some investigators (2).

Egyptian blue is a beautiful blue dyestuff which was widely used for this purpose in Egypt during the time of the IV dynasty, as well as in the Roman Empire. According to Vitruvius it has been invented in Alexandria and prepared in special factories from copper saw dust, sand and limestone with additional fluxes. In the Middle Ages the secret of manufacturing Egyptian blue was lost and therefore it is at present quite unknown in industry. Specimens of ancient painting have been preserved up to the present days, in which this wonderful pigment had been used, e. g. some most beautiful frescoes of the Vatikan. This mineral has been frequently found in Pompei and it has been also discovered in France in the Gallic-Roman tombs.

The exceptional resistance of the Egyptian blue to weathering agents (explaining the excellent preservation of the paintings made with this pigment

tens of centuries ago) prompted many investigators to give much attention to its study and synthetic production. In foreign literature a number of papers are devoted to this problem, showing the comparative facility of its production and giving more or less detailed descriptions of the mineral, however, the recipes of its preparation are mostly lacking.

The present authors also have made attempts to synthesize this mineral and the results have been quite satisfactory. Egyptian blue is easily produced from metallic copper, copper oxide, carbonate or acetate by sintering with $CaCO_3$ and SiO_2, taken according to the formula with an addition of alkali carbonate as a flux. In our experiments sintering occurred at 850° for 20 hours. The output of Egyptian blue was in all cases above 50% of the charge by weight.

Thus, we may confidently speak of the possibility of Egyptian blue manufacturing on a large scale for practical purposes. The exceptional properties of this mineral dyestuff will undoubtedly lead to its wide application in the national economy and in art, particularly in monumental painting widely developed in the Soviet Union.

At present the authors are engaged in a detailed study of the chemical composition of Egyptian blue and of the specific conditions of its formation, and in the nearest future they intend to publish a paper on this subject.

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AGROCHEMISTRY

EFFECT OF NITROGEN-POTASSIUM PERIODIC SUPPLY UPON YIELD OF ROOT AND STORE OF RUBBER IN KOK-SAGHYZ

By A. F. KALINKEVICH

(Communicated by D. N. Prianishnikov, Member of the Academy, 26.VII.1938)

Requirements of a plant to its environment vary with respect to different stages it is going through during life. Even within «the same stage different environmental conditions are needed for the development of different organs (e. g. leaves and roots)» (1).

On the ground of this statement experiments have been carried out by the author in which the highest yields of the rubber both with regard to quantity and quality were obtained when the plant during the period of energetic formation of root and accumulation of rubber enjoyed quite another supply of nutrients compared to the preceding period of rosette formation, flowering and fruiting.

The data referred to in Table 1 are extracted from an vegetative experiment of 1937 on the periodic mineral supply to kok-saghyz. They show the effect a suitable change of nitrogen and potassium supply levels against the period of plant's life has upon the yield of root and rubber. An increase of nitrogen and potassium rations within the last one and one and a half months of vegetation, i. e. after mass fruiting, promotes the production of leaf at the expense of root, i. e. to the detriment of both the quantity and quality of the rubber. Variants 1—2 and 4—7 display differences not only with respect to the amount, but also biochemical process in leaves which will be discussed farther on.

One more statement of great importance may be inferred from Table 1: variants with reduced nitrogen supply within the stage of mass fruiting produce the highest yield both in percentage and absolutely, provided they were supplied with nitrogen sufficiently during the preceding period. This general statement concerning the supply of minerals to kok-saghyz has found support in our experiments of 1935, 1936 and 1937. The latter have proved that the erroneous conclusions, some of the authors (2) were led to, were due to their having not differentiated the supply according to periods. They believed budding and outset of flowering to form a critical period limiting the aggregate store of rubber in kok-saghyz, whereas in our experiments we induced both different ratio and absolute yield of rubber by differentiating the supply during the last period of vegetation, this being achieved in plants which had enjoyed equal supply levels during their preceding «history» till the close of mass fruiting. Questionable and lacking evidence is also the statement advanced (2) that a rich supply of nitrogen to kok-saghyz,

Table 1

№	Supply conditions						Dry weight of 100 plants at harvesting 4.X.1938			Rubber			
	Planting—formation of rosette 27.IV—12.VI		Formation of rosette—fruiting 12.VI—1.VIII		Close of fruiting—harvesting 1.VIII—4.X		Leaf	Root	Plant	% of rubber	% of resin	rubber / resin	Absolute yield in mg per 100 plants
	N	K	N	K	N	K							
1	1/3	2	2	2	2	2	156.6	216.0	372.6	3.5	1.7	2.06	7560
2	1/3	2	2	2	1/3	2	55.5	270.0	325.5	6.6	2.4	2.75	17820
3	2	2	2	2	2	2	264.0	295.5	559.5	4.1	1.7	2.41	12095
4	1/3	1/2	2	1/2	1/3	1/2	48.0	284.4	332.4	4.9	2.1	2.33	13936
5	1/3	1/2	2	1/2	2	1/2	208.9	289.8	498.7	5.2	1.9	2.74	15080

Note: 1) Figures 1/3, 1/2, 2 denote rations of nitrogen or potassium in terms of Hellriegel's mixture. 2) Experiment was done in sand cultures in 8 variants, against the background of Hellriegel's medium. Capacity of vessel, 10 kg sand. Three plants were left in each vessel.

until budding and flowering have set in, lowers the aggregate yield of rubber in the plant. As the period of budding and flowering is preceded by the formation of leaves, the lack of nitrogen within this period will considerably reduce the leaf area, and we know that no good yield of seed, root or rubber may be obtained without a sufficient amount of leaf formed in due time (before flowering).

Our experiments concerning the question which have being carried on for two years lead us to the following conclusion. In order to secure highest yield of seed and rubber in kok-saghyz it is indispensable to bring the growth of leaf to its maximum before mass flowering has set in and afterwards to preserve the leaf in such a way that it should profit the root.

Special attention should be paid to the influence of potassium upon the yield of rubber since some of the authors assert that potassium fertilizers have proved unfavourable in this respect ([3], [4], [5]). Our experiments have clearly shown that a suitable dosage of nitrogen with regard to time (according to periods) is the most important condition of the positive effect of potassium upon content, absolute yield and quality of the rubber.

The efficiency of potassium nutrition is greater, if nitrogen is supplied within the period of intensive root-formation and rubber storing in less quantity than it was the case during rosette-formation and fruiting.

But if at the end of vegetation (just before harvesting) nitrogen is supplied alongside of potassium in greater quantities, the growth processes (leaf production) and the synthesis of protein substances are promoted to the detriment of the root as well as quantity and quality of rubber.

The data referred to show that cases are possible when some unfavourable factors (in our experiment, insufficient supply of potassium) may result in that a comparatively large supply of nitrogen at the end of vegetation will not reduce the yield of rubber. In our case this should be put

down to the ageing of leaves going on more quickly because of the lack of potassium.

The effect of periodic mineral supply to kok-saghyz is linked with the change of biochemical processes taking place in separate organs of the plant (Table 2, 3).

Table 2

Conditions of supply						% of nitrogen per dry matter, 4.X				mg of protein nitrogen per 100 plants			mg of protein nitrogen in leaf per 1 mg of rubber
24.IV—12.VI		12.VI—1.VIII		1.VIII—4.X		Leaf		Root		Leaf	Root	Plant	
N	K	N	K	N	K	Protein	Total	Protein	Total				
1/3	2	2	2	2	2	2.85	3.47	1.08	3.73	4 463	2 833	6 796	0.60
1/3	2	2	2	1/3	2	1.30	1.43	0.89	3.44	721	2 403	3 124	0.04
2	2	2	2	2	2	2.30	2.93	0.85	3.46	6 072	2 511	8 583	0.50
1/3	1/2	2	1/2	1/3	1/2	1.55	1.67	0.57	2.22	744	1 621	2 865	0.05
1/3	1/2	2	1/2	2	1/2	2.34	2.94	0.90	3.22	4 988	2 608	7 596	0.33

The investigation carried on by the author has revealed a greater content both of protein and total nitrogen in the leaves of those plants which had been given more nitrogen at the end of vegetation, i. e. within the period of intensive formation of root and storing of rubber; while less nitrogen has been found if the supply was reduced within this period.

The synthesis of protein substances is promoted to a still greater extent if potassium alongside of nitrogen is given in larger quantities during that time. But there appears a checking of growth processes and of the synthesis of protein substances when nitrogen supply is reduced against the background of an increased supply of potassium (Table 2). Attention should also be given to quantity of protein nitrogen in the aggregate mass of leaf at harvesting.

The figures of Table 2 show a considerably greater absolute quantity of protein nitrogen in the leaves of those variants which were being given more nitrogen within the period of mass fruiting.

The ratio of the amount of protein nitrogen in leaf to the absolute quantity of rubber shows the same dependence: the lowering of nitrogen supply level within the period of vegetation causes the ratio of the absolute amount of protein nitrogen in leaf to the yield of rubber to go down, and conversely.

This is an evidence of that the intensified synthesis of protein substances within the last period of vegetation is going on to the detriment of rubber accumulation.

The change of nitrogen or potassium supply to the plant tells upon the content of carbohydrates in its separate organs. In Table 3 data are given on the content of carbohydrates (reducing sugars and saccharose) in the leaves and roots of kok-saghyz at the time of harvesting.

The conditions of nitrogen supply influence the transportation and redistribution of carbohydrates between separate organs in the plant.

When the plant is given more nitrogen within the period of intensive root formation and rubber storing (just after harvesting), there are being created conditions under which a great expense of plastic substances (say

Table 3

Conditions of supply						Leaves			Roots		
24.IV—12.VI		12.VI—1.VII		1.VII—4.X		Reducing sugars %	Saccharose %	Soluble sugars %	Reducing sugars %	Saccharose + insulin, %	Soluble sugars %
N	K	N	K	N	K						
1/3	1/2	2	1/2	1/3	1/2	4.77	5.18	9.96	0.27	51.66	51.93
1/3	1/2	2	1/2	2	1/2	9.48	5.35	14.84	0.12	32.08	32.21
1/3	2	2	2	1/3	2	2.05	6.83	8.89	0.20	51.08	51.29
1/3	2	2	2	2	2	3.91	5.19	9.11	0.10	42.30	42.40

carbohydrates) involved in growth processes goes along with a slowing down of the movement of sugars from the leaf and a reduction of their content in the root. Lowering of nitrogen supply favours the storing of sugars in the root.

The ratio between nitrogen and potassium supplies exerts a certain influence upon the content of definite forms of sugars in the plant, especially in its leaves. The more nitrogen the plant is given, the higher the content of reducing sugars, while potassium increases the content of saccharose (sugar after five minutes of hydrolysis).

This particular instance illustrates the influence which the ratio of separate supply elements exerts on biochemical processes in plants ([6], [7]).

Conclusions: 1) The process of rubber storing is favourably promoted by an increase of nitrogen supply during rosette-formation—fruiting with its subsequent decrease at the end of vegetation. This is the most important condition for the rise both of relative and absolute yield of rubber and of its quality.

2) A suitable dosage of nitrogen supply with regard to periods of vegetation proves of great importance for the positive effect of potassium upon the yield of rubber in kok-saghyz.

3) Age variations of individual organs, especially leaves, should be controlled by changing the supply of nitrogen according to life stages of kok-saghyz. When this plant is given more nitrogen from the beginning of rosette-formation till mass fruiting, its leaves are forming intensively. Afterwards, however, i. e. within the period of root formation and rubber storing, the supply of nitrogen should be reduced to prevent rapid ageing of the leaves. Such a leaf is characterized by checked synthesis of protein substances and the use of assimilates for other biochemical processes.

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BIOLOGY

SUSCEPTIBILITY OF THE SPOROGONY PROCESS IN OÖCYSTS OF COCCIDIA TO ULTRA-VIOLET RAYS AS DEPENDENT UPON TEMPERATURE CONDITIONS AND THE REPARATIVE POWER OF OÖCYSTS

By G. M. LITVER

(Communicated by N. V. Nassonov, Member of the Academy, 21.VII.1938)

The cycle of sporogony is closely connected with the temperature of the surrounding medium. The rate of metabolic processes in oöcysts increases with the rise of temperature, the same as in other cells. We seem to face here a phenomenon, analogous to the one occurring in the chemist's flask, where usually with a rise of temperature by 10° the rate of chemical reactions increases twofold.

The main task of the author has been to determine whether the change of temperature conditions of the medium has any bearing upon the effect of radiant energy on processes of sporogony or the variation of the temperature factor makes no difference at all.

Radio-biology has accumulated a great deal of experimental material concerning the effect of radiant energy on various biological objects depending upon temperature conditions of the surrounding medium, but unfortunately there is a great divergence of opinion on this question among investigators, and it is difficult at the present time to make any generalizations whatever.

Those rather significant contradictions existing among investigators regarding the effect of rays under different temperature conditions of the environment are due to many reasons. Apart from the different procedures used by the authors and different objects of observation, frequently a very important circumstance has been neglected which was observed as early as 1918 by Bovey and Klein, namely, that irradiation is not only affected by change of temperature, but «radiant energy makes the organism susceptible to temperature».

Taking into account the observations of Bovey and Klein, we thought it necessary to organize on a wider scale experiments envolving treatment with ultra-violet rays, the effect of the temperature factor on the processes of sporogony being different in each case. Our experiments may be classed among three principal series. In cases referred to the first series suspensions of oöcysts *Eimeria magna* were placed before exposure for 3 hours into water baths with different temperature (from + 26° to —1°); then the oöcysts were exposed to radiation under ordinary room conditions (15—16°). The process

of sporogony was taking place in a thermostat at temperature+ 27°. The second series of experiments consisted in the irradiation of suspensions of oöcysts at different temperatures (from+26 to —1°), whereupon they were placed in a thermostat with $t°$+27°. And finally, in the third series of experiments oöcysts treated at room temperature were kept at $t°$+26° to—1°, and then placed in a thermostat with $t°$+27°C.

The experiments of the last series were the most complicated as it was necessary to keep the oöcysts exposed to low temperatures for various periods before placing the culture in a thermostat.

To examplify the aforesaid: when oöcysts are kept at temperature—1° to + 4°, usually no development takes place, but as soon as such oöcysts are placed under normal conditions, sporogony begins.

It follows therefrom that irradiated cultures may be kept at low temperatures over any length of time without develop and only thereafter, when placed under favourable conditions, they can be made sporulate.

Our data have shown that a pretreatment of oöcysts for a period of half an hour to 3 hours with temperature —1° to + 20° C did not influence the susceptibility of oöcysts to ultra-violet rays. Whereas, if oöcysts were kept prior to irradiation at temperature +26°, the ratio of destruction would increase. What is the explanation of the results obtained?

We are inclined to refer the high percentage of destruction of oöcysts kept prior to irradiation for 3 hours at temperature +26° to the fact that ultra-violet radiation was acting at another stage of development. Observations with a microscope have shown the appearance of pigmented clusters in the centre of the plasma globule of such oöcysts, i. e. the beginning of intensive development, while in other oöcysts no morphological changes could be detected.

Moreover, a number of experiments (the second series) have shown that if oöcysts in exactly the same stage of development are exposed to irradiation at different vital temperatures, no differences whatever in the ratio of destruction are to be found.

Let us proceed to the next series of experiments envolving the rôle of temperature conditions in the process of sporogony after irradiation. Suspension of oöcysts irradiated at 14° was placed in special thermostats with different temperatures, whereby cultures kept at temperature from +17 to +26°C remained there until the end of sporogony. As to cultures kept at temperature from +8° to —1°, the duration of the period varied from 6 hours to 18 days, whereupon they were placed in a thermostat with the usual temperature (+37°). First of all, let us take note (Table 1) that the keeping of cultures after irradiation throughout the entire period of sporogony under various conditions of temperature within +14° to +26°C has no effect on the percentage of oöcysts killed.

Table 1

The Effect of Different Temperatures after Irradiation

Experiment	Temperature °C	Radiation dosage		Duration of sporogony in control
		2 min.	4 min.	
		Percentage of destruction		
a	26	48.8	90.0	62 hours
b	20	49.1	89.1	70 »
c	14	48.8	86.9	88 »
d	8	24.6	43.2	18 days

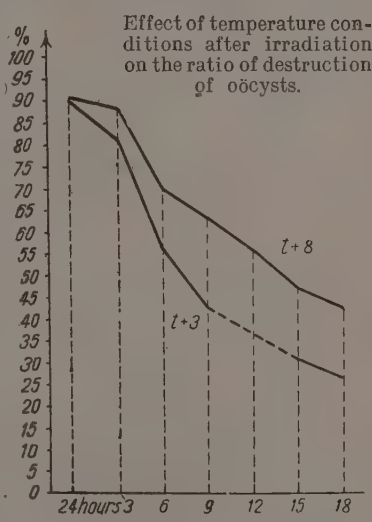
However ,different results may be obtained when oöcysts have been subjected to relatively low temperatures (from —1 to +8°), and only thereafter placed under usual conditions of the thermostat (Table 2).

Table 2

The Effect of the Temperature Factor after Irradiation upon the Ratio of Destruction of Oöcysts

Experiment	Temperature, °C	Duration of exposure of oöcysts to temperature effect after irradiation						
		24 hours	3 days	6 days	9 days	12 days	15 days	18 days
		Per cent destruction						
a	+3	90.4	88	70	63	56	47	43.4
a_1	+3	90.1	81.3	56	42.9	Not counted	31.7	26.1
a_2	0 to—1	90	81.2	56.1	42.1		32	25.4

The curve («a» see figure) shows that at a temperature of 8° the longer oöcysts have been subjected to said temperature, the less the number of undeveloped oöcysts. For instance, keeping oöcysts within 24 hours after irradiation leads to destruction of 89%. Whereas 18 days of action of the same temperature destroys only 43% of them. Such regularity is still more emphasized in experiments «a» and «a_2» (Table 2). What should be the explanation of this phenomenon?



Effect of temperature conditions after irradiation on the ratio of destruction of oöcysts.

Duration of exposure of oöcysts to the action of temperature after irradiation.

It seems to us that the explanation of the regularities present may be found in the comparison of the ratio of destruction of irradiated oöcysts and of conditions determining the rate of sporogony.

Experiments have shown that when oöcysts were subjected to the influence of various temperatures before, during or after irradiation, provided the rates of development were about the same, these temperatures had no bearing upon the percentage of oöcysts killed.

Experiments «a», «a_1» and «a_2» (Table 2) bear evidence that in order to slacken the lethal effect of ultra-violet rays, it is necessary to subject oöcysts after treatment to a prolonged action of low temperatures.

But what is the essential effect of various vital temperatures on processes of sporogony?

From data by many authors (Pérard, Reichenow and others) one may see that at temperature from +3 to —1° oöcysts survive, though no sporogony is observed.

What is the nature of this phenomenon as shown by our experiments?

Ultra-violet rays, affecting the plasma globule of oöcysts, produce certain changes in the «live substance», whereupon such oöcysts are kept either at +26 to +14°C, or at +8 to —1°.

In the former case oöcysts should sporulate, but microscopic observation has shown that the majority of them fail to sporulate normally, malformations arise which succumb in the end. In the latter case the same oöcysts have remained over a long period under conditions inhibiting their development. Consequently, during the period of rest oöcysts undergo recovery (reparation) of changes produced by ultra-violet rays.

Thus, our experiments have shown that the longer the period of rest, the completer it is (lower temperature), the greater the number of forms recovering from injury caused by ultra-violet rays.

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BIOLOGY

THE SIGNIFICANCE OF AEROBIC AND ANAEROBIC CONDITIONS FOR THE EFFECT OF ULTRA-VIOLET RAYS ON PROCESSES OF SPOROGONY OF COCCIDIA FROM RABBITS

By G. M. LITVER

(Communicated by N. V. Nassonov, Member of the Academy, 21.VII.1938)

It is known that oöcysts of coccidia fail to develop in the absence of oxygen, but their viability continues for a long time; when transferred into aerobic conditions they sporulate normally.

If our suggestion that the reparative power of oöcysts treated with ultra-violet rays is in inverse relation to the rate of development or the period of rest between exposure and the beginning of sporulation is correct, then, consequently, by placing treated oöcysts under anaerobic conditions, it is possible to reduce the percentage of forms failing to develop. In case no such reduction is observed in regard to treated oöcysts placed for a continuous time in an atmosphere of hydrogen, our original statement should be reconsidered. To clear up the foregoing we have carried out a series of experiments.

Besides, experiments with the effect of ultra-violet rays on processes of sporogony under aerobic and anaerobic conditions are of interest, insofar as they permit to solve the problem as to the connection between the effect of ultra-violet rays and the disturbance of oxidation processes in oöcysts.

Some Peculiarities in Methods of Investigation. The effect of anoxybiotic conditions on processes of sporogony is usually studied by placing oöcysts in the Omelyansky-apparatus, or in glass chambers filled with hydrogen. If glass was easily transmitting ultra-violet rays, our task would be a simple one. But the capacity of glass to absorb ultra-violet rays induced us to devise a special anaerobic chamber for ultra-violet radiation. Our chamber excelled other similar apparatus in that it made it possible to create in the liquid medium more perfect anoxybiotic conditions.

In the majority of experiments hydrogen was passed through the chamber 8 hours prior to irradiation. Oöcysts * placed in the apparatus at the stage of a plasma globule, as controls have shown, failed to develop during that period of time. We have never succeeded to detect in the centre of the plasma globule even a small accumulation of pigmented clusters, which proves that the anoxybiotic conditions were sufficiently controlled. In the first series of experiments we placed oöcysts which were at the stage of plasma globules for a few hours (from 6 to 24) under anoxybiotic conditions, to expose

* Of the species *Eimeria magna*.

them next to the action of a quartz mercury vapour lamp in the presence of oxygen.

Table 1 shows the results of these experiments.

Table 1

The Effect of Ultra-Violet Rays on Oöcysts Previously Placed under Anoxybiotic Conditions (exposure with inlet for oxygen)

Period the oöcysts were kept under anaerobic conditions previous to exposure	Period of treatment	
	2 min.	4 min.
	Per cent undeveloped oöcysts	
6 hours	46.1	87.6
12 »	46.3	87.1
18 »	47.0	87.6
24 »	46.7	87.4
Control	46.4	87.3

From the data obtained it may be stated that by depriving oöcysts of oxygen before irradiation no effect is produced on the ratio of their viability.

The second series of experiments consisted in keeping oöcysts, which were at the stage of a plasma globule, before irradiation (within 24 hours) and at the moment of irradiation in the atmosphere of a current of hydrogen. Immediately after exposure the oöcysts were placed under aerobic conditions. Simultaneously controls were performed involving oöcysts treated under aerobic conditions.

As shown in Table 2, where the results of these experiments are summarized, we failed to obtain any material difference between experiments and controls.

Table 2

Period of exposure and of subjecting oöcysts to anaerobic conditions during exposure	2 min.	4 min.
	Per cent undeveloped oöcysts	
Period of subjecting oöcysts to anaerobic conditions before exposure	4 hours	47.1	87.6
	8 »	46.8	88.0
Control	—	46.3	87.5

The following experiments were carried out in order to find out the effect of placing oöcysts after exposure under conditions of anoxybiosis. To solve this question two variants of experiments could be tried: either to expose oöcysts in hydrogen, leaving theme subsequently under the same conditions over different periods before admitting the current of oxygen, or to irradiate oöcysts under anaerobic conditions, but subject them after radiation over a certain period of time to anoxybiosis, to be followed by inlet of oxygen.

Our work included both variants.

Experimental. For the first variant of the experiment 10 cultures of oöcysts *Eimeria magna* have been exposed to irradiation at the stage of a plasma globule under anaerobic conditions, then one of them (set «a») was subjected immediately after irradiation to the current of oxygen—this culture served as control.

Other cultures of oöcysts were subjected to the oxygen current at the following intervals after exposure: 24 hours, 3, 6, 9, 12, 15, 18, 21 and 24 days.

The second variant of the experiment was analogous to the first one, except that the cultures treated with ultra-violet rays were immediately thereafter placed under anaerobic conditions.

The subsequent supply of oxygen was effected at the same intervals as in the first experiment.

Both experiments yielded similar results, therefore Table 3 contains data only of the first variant.

Table 3

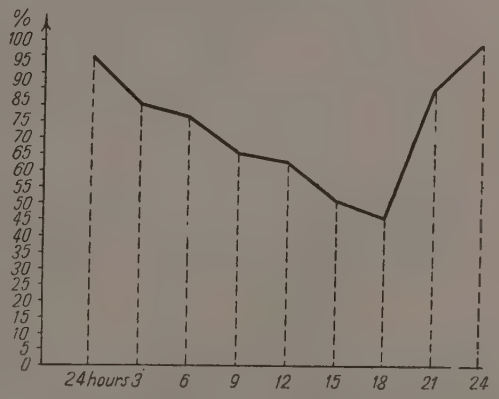
The Effect of Anaerobic Conditions after Treatment with Ultra-Violet Rays on the Mortality Percentage in Oöcysts

Period of subjecting oöcysts to anaerobic conditions after exposure	24 hours	3 days	6 days	9 days	12 days	15 days	18 days	21 days	24 days
Period of exposure	Per cent undeveloped oöcysts								
4 min.	94.2	80.1	76	66.3	62.6	51	46.1	86	100
Untreated oöcysts under the same experimental conditions	0.15		0.2		—	—	—	88	100

The data of the above table served as material for curve (see figure).

Analysis of the curve obtained shows that by placing oöcysts after exposure under anoxybiotic conditions for a period from 24 hours to 18 days, it is possible to reduce the mortality percentage due to the effect of ultra-violet rays. But if the oöcysts remain over a more continuous time under conditions of anaxybiosis, the ratio of mortality increases, and up to the 24th day all of them perish. What is likely to be responsible for the phenomenon observed?

Effect of anaerobic conditions after irradiation upon the ratio of destruction of oöcysts.



Duration of exposure of oöcysts to anaerobic conditions after irradiation.

It seems to us that by placing oöcysts at the stage of a plasma globule into the atmosphere of hydrogen conditions are created which are closely approaching those which are operative at low temperatures. In both cases no development follows but oöcysts remain viable for a long time. True enough, we are not inclined to state that the operative mechanism is the same in both cases. On the contrary, under the influence of cold all reactions in oöcysts are probably stopped in a like degree, whereas in the absence of oxygen the first link in the cycle of sporogony—the division of the plasma globule—is eliminated, the subsequent stages of development arising only as a continuation of this link. Thus, though the mechanism operating in both cases is not alike, it leads to identical results—to failure of development of oöcysts. However, it is a well known fact that such oöcysts remain viable for a long time: metabolic processes undoubtedly proceed in

them, which may be seen from their ability for further development, as well as from the analogy with eggs of nematoda, the latter having been conclusively proved by Holthusen.

Our data justify the suggestion that during the period of delay of development as a result of anoxybiosis there occurs a slow recovery from injuries caused by ultra-violet rays. And the longer the period of rest within definite limits, the more complete the reparation. The high percentage of oöcysts perishing on the 24th day under anaerobic conditions is not due to the effect of ultra-violet rays, but depends on the general ability of oöcysts to stand no more than 25 days of anoxybiotic existence. This can be easily proved by keeping exposed oöcysts under conditions of a hydrogen chamber during various periods of time (Table 3—control).

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BIOLOGY

ON THE GRADIENT-FIELD

By M. SAVCHUK

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The theory of fields and gradients arose in the XX century, its further development being connected with such names as those of Bovern, Childe, Weiss, Gurvitch, Spehmann and others. Later on to both these notions the term «gradient-field» was applied (Huxley). According to the above authors, the differentiation of organs in the course of their development is determined by morphological fields or the gradient-fields.

The adherents of this conception are backing their theory with a series of observations made on regeneration processes. Thus, according to some authors, a graft of a regeneration blastema of the anterior extremity at an early stage of development to the stump of the hind extremity results in the regeneration of the hind extremity. When transplanting a regeneration blastema of the tail at an early stage at the place of an amputated extremity, the latter and not the tail regenerates. The supporters of the gradient-field theory explain this phenomenon by the fact that the cells of the regenerating blastema are multipotent at an early stage and their differentiation is determined by the gradient-field. This point of view is also supported by experiments on transplanting extremity discs within the zone of activity of the gradient-field. According to Helmich, Detwiller and others such transplantates are subject to resorption.

In order to check these theories we undertook the following experiments.

1) We repeated on newts and axolotls the experiments with the transplantation of the early stages of the regenerating blastema of the fore leg at the place of the amputated hind leg. Aside from this, we grafted the tail blastema at the place of the amputated extremity and made a series of supplementary operations and observations.

To gain a clearer idea of the occurring processes we combined two races of axolotls, the white and the black ones. These experiments are described in more detail elsewhere; therefore, when dealing here with the character of the cells of the regenerating blastema, we shall but briefly point to the results obtained.

a) The cells of the regenerating blastema are not multipotent at any stage; even at early stages they are relatively differentiated.

b) The formation of the hind leg in the case of transplantation of the regenerating blastema of the fore leg, or the transplantation of the tail blastema at the place of the amputated leg is not caused by the influence of the field, affecting in a certain way the differentiation of the cells of the graft, but it is due to the fact that these cells are subject to resorption and either

do not play any rôle in the formation of the regenerate, or this rôle is quite insignificant, regeneration taking place at the expense of the tissues of the host. Similar results are obtained when transplanting extremity discs. If a sufficiently thin disc is transplanted, the regeneration occurs at the place

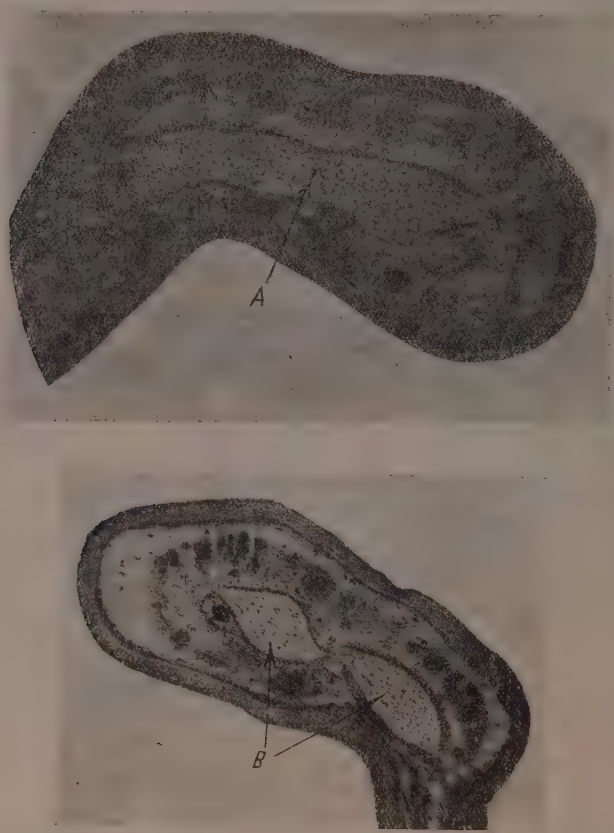

Fig. 1. A cross-section of the regenerate, formed at the place of the amputated leg, in whose formation both tail and leg tissues took part (*A* and *B*—osseous part of the regenerate). (Experiments § 2.)

of transplantation; if, on the other hand, the transplantate is involved in the formation of the regenerate, a chimera is obtained.

The results of our experiments are demonstrative enough not to allow us to consider the regenerating blastema as a complex of quite uniform multipotent cells, which differentiate later on under the influence of the field.

2) At the place of exarticulated extremities of newts and axolotls we transplanted bits of tissue from the tail and the legs. The regenerates assumed various malformations, consisting of tail or leg tissues. The skeletal part assumes most various shapes, the tail and the extremity sometimes regenerating separately, which is described by us elsewhere.

3) At the place of the exarticulated leg of a newt or an axolotl we transplanted tail discs in a way that they did not cover the whole wound surface. After such operations sometimes occurs the regeneration of a malformed

organ, while sometimes the extremity and the tail are regenerated separately.

4) At the place of the amputated extremity we transplanted leg discs so that the graft did not cover the whole wound surface. In such cases the regeneration of two and more extremities may take place within the same field—from the graft and the parts of the wound surface left uncovered.

5) To the stump of the amputated legs of a newt, below the knee, we transplanted discs from the proximal end of the leg. The ingrown grafts regenerated in the distal part, which resulted in the formation of an abnormally long leg with two knees. When the graft did not cover the whole wound surface of the amputated leg, regeneration also took place from the wound surfaces left uncovered. Similar results were obtained when transplanting the blastema of the tail and extremities to the stump of the leg. (If the tissues of the blastema do not become resorbed, regeneration occurrs at the place where the graft had been extracted, and not at the place of transplantation).

Fig. 2. Regeneration of three extremities in the same field. At the place of the amputated left hind leg of a newt was transplanted a disc of a leg cross-section thus that the graft covered the middle of the wound surface. Regeneration proceeded both from the graft and from the two sections of the wound surface (formed by the exarticulation of the leg) uncovered by the graft. (Experiments § 4.)

Conclusions

The theory of fields and gradients or of the «gradient-field» is in its essence an idealistic theory. With some authors (Gurvitch and others) it has an openly idealistic character, while with others its idealism is veiled.

This theory is based on isolated random phenomena or on an erroneous interpretation of the experimental data. For instance, the regeneration of the leg after a transplantation of the tail blastema at the place of an amputated leg, is wrongly interpreted by some investigators, who consider the regeneration from the host's tissue as occurring from the graft. Our experiments have shown with sufficient clearness that the tail tissues cannot form an extremity. The character of the regenerated organ depends on the regenerated tissues and the place of the graft does not affect the form-building capacity of the regenerate, with respect to a possible formation of a leg from tail tissues. The place or the «field» plays a part only as far as we understand under the term «field» the complex of tissues, corresponding to a definite form. In the reverse case the notion of the «field» is nothing but an idealistic abstraction, leading to an erroneous understanding of the processes of development. The conclusions of Helmich, Detwiller and others that two legs cannot regenerate within the zone of action of the gradient-field are erroneous too, our experiments having shown that such an assumption lacks evidence.

Undoubtedly there exists a certain gradation of tissue differentiation connected with different processes in the organism. Thus, for instance, from the wound surface of any level of the leg or tail only the distal end regenerates (the same is true for the transplantation of discs). The median part of the tail also differs in this respect from its root and end, though this fact does not depend on any «gradients», which are external with regard to the organism, but is connected with the structure of the tissues and their relations and interaction.

Thus, the theory of fields and gradients such as that of Bovern, Weiss, Gurvitch, Huxley and others is based on misconceived experimental data, the result of development being considered by its adherents as the cause of the development.

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GENETICS

TIME AND SPONTANEOUS MUTATION IN *DROSOPHILA MELANOGASTER*

By N. I. SHAPIRO

(Communicated by A. A. Sapehin, Member of the Academy, 10.VII.1938)

The present communication gives material on the study of the dependence of the spontaneous mutation rate in *Drosophila melanogaster* on the length of life of cells, in which hereditary changes have occurred. It is of interest to find out what kind of dependence there exists berween mutation and the duration of life of these cells. A series of theoretical questions are connected with this problem. Some of them will be discussed below. It is necessary to point out that the solution of this question is no less important for the understanding of the nature of spontaneous mutations, than the problem of dependence of mutation rate on X-rays dosage for the understanding of the nature of X-rays mutations. In fact the same questions are studied in both cases. Although we do not know as yet what cause or causes determine the frequency of natural mutations, the study of mutation rate in cells with different duration of life will help us to find out the dependence of natural mutation rate on different dosages of mutagenic factors acting in nature. The question of the dependence of mutation frequency on the period of time the cells have been kept, was carefully studied in different plants (*Crepis*, *Datura* and others). It has been shown in a number of works that the frequency of mutations raises with the increase of the length of time during which seeds and pollen were kept ([1],[7],[9]). The character of this dependence was, however, not determined. It is as yet not found out whether we are dealing here with a direct proportionality, or there exists some other kind of dependence. The solution of the problem is very important for the understanding of the nature of mutation. The study of this problem in *Drosophila melanogaster* would give a more detailed picture of the dependence of mutation on the factor of time. The number and type of arising mutations (gene mutations, chromosomal aberrations) can in the best possible way be studied in *Drosophila melanogaster*. In the present communication we do not give data on the kind of dependence of mutation rate on the duration of life of the cell. It will be the subject of our further investigation. We restrict ourselves here to the demonstration of the very existence of such dependence.

The frequency of sex-linked lethal mutations in *Drosophila melanogaster* was studied. In one case mutation rate in males which developed during 12 days at 25°C was studied. In the other case not only the number of mutations arising during the period of development of the males, but as well

mutations arising during the time of keeping the sperm in spermathecae of females were studied. The ageing lasted 30 days at a mean temperature 14°C. The *ClB* method was applied. Data obtained in the experiments are given in the table.

Frequency of Spontaneous Mutation in Spermatozoids at Different Age

Time of keeping of spermatozoa in female spermathecae in days	Temperature at which mutations arose, °C	Number of chromosomes studied	Number of sex-linked lethal mutations	Percentage of arising sex-linked lethal mutations	Percentage of arising sex-linked lethal mutations after the correction for temperature conditions	$M_{diff} \pm m_{diff}$
0	25	6 852	18	0.26	0.26±0.05	0.34± ±0.12
30	14	5 580	24	0.43	0.60±0.10	

The data of the table show that the percentage of mutations arising in the experiment, in which spermatozoa were kept in spermathecae of the females, is one and a half—twice as great as in the experiments in which spermatozoa were not aged. The difference of figures characterizing the rate of mutation in the two experiments is even greater than was found experimentally. It is necessary to point out that the ageing was carried out at a temperature of about 10°C lower than the temperature at which flies were developing. If we make the necessary correction, we shall obtain a considerably higher percentage of arising mutations in spermatozoa that were kept during 30 days. The attempt to establish the coefficient of temperature (Q_{10}) for the process of mutation in *Drosophila melanogaster* was made in two works. In the first one ([5]) Q_{10} was found to be equal to 2—3. In the second work made under better controlled conditions Q_{10} was equal to about 5. If we take the minimum value of Q_{10}, we must assume the percentage of arising mutations in spermatozoa after ageing to be as high as 0.60% (0.43—0.26=0.17; 0.17×2=0.34; 0.34+0.26=0.60%).

The comparison of mutation rate in both series after this correction shows that the probability of a statistical significance of this difference is high enough. It would be still greater if the value of Q_{10} would exceed 2. As was said above, we have the right to do this, as there are reasons to believe that the value of Q_{10} for the process of mutation is higher than 2. Taking the lowest magnitude of Q_{10} we have therefore chosen the most unfavourable conditions for proving the existence of influence of time upon mutation.

It is therefore obvious that data presented in this paper give evidence in favour of the important rôle of time in the spontaneous process of mutation. Similar results were earlier obtained in *D. melanogaster*, however, by a different method. The agreement of results in two independent experiments definitely proves that the number of arising mutations depends on the duration of life of the cells, in which mutations are studied.

Several conclusions may be drawn on the basis of the facts observed.

1. The existence of a continuity in the process of mutation. Comparing the fact of existence of a dependence between mutation rate and the age of the cell with the results obtained in numerous experiments by different

authors, who invariably found during many years approximately the same rate of arising mutations in *D. melanogaster*, we may conclude that the process of mutation is connected with no definite periods of life of the species (or separate populations) ([8]) and proves to be continuous.

2. We should expect a greater number of arising mutations in old animals. This was already pointed out before ([4]).

3. The rôle of time in the evolution of mutability. This question was discussed by us in a special paper. We shall therefore touch upon one point only. If we consider the existence of dependence between mutation rate and the duration of cell-life, and the difference in the time necessary for the development of one gamete generation in different plants and animals, we should expect a proportionally greater number of mutations in species with a long life cycle. In other words, the number of mutations arising per one gamete generation would be greater in organisms with a long cycle of development and smaller in organisms with a short cycle of development. However, some considerations ([6]) and facts ([3]) prove the non-existence of connection between the length of one gamete generation and the number of mutations arising during this period. Species with different length of developmental cycles have about the same values characterizing the rate of mutations per gamete generation. We can therefore conclude that if we calculate the number of arising mutations not per gamete generation but per a general unit of time (for instance a year), the rate of mutation will be quite different in different species. In other words, it is the number of mutations per unit of time that changed in evolution. Hence in species with a long life cycle the number or mutations per unit of time is smaller than in species with a comparatively short cycle.

4. The accumulation of mutations in natural populations of *Drosophila*. This question is connected with the probable rôle of hibernation of *Drosophila* in the accumulation of mutations in populations. It is known that after the period of propagation during summer, *Drosophila* spends the remaining part of the year in hibernation. The length of hibernation varies under different climatic conditions. In many regions the length of hibernation is somewhat longer than that of the period of propagation and in any case much greater than the time necessary for the development of one generation under optimal conditions. Since mutations arise in proportion to the time of life of the cells, many mutations will accumulate during hibernation. If the frequency of mutations for the entire genome of *Drosophila* in one gamete generation is about 4½%, then the number of mutations accumulated during hibernation must exceed this value as many times as the length of hibernation exceeds the duration of development of one generation. Temperature conditions, however, should be taken into account. As the temperature during hibernation is much lower than under normal conditions for which this rate of mutability was studied, the rate of accumulation of mutations will be much lower. It is, however, necessary to remember that sufficiently low temperature increases the rate of mutation ([2]); fluctuations similarly increase the frequency of mutation ([10]) as compared with constant temperature conditions. It is therefore obvious that in many cases we should expect a much greater accumulation of mutations during hibernation than during one gamete generation in summer. This latter statement can be tested experimentally, especially for sex-linked mutations. We should expect that a population after hibernation is richer in mutation than in the subsequent period, i. e. after the action of natural selection.

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GENETICS

INTERSPECIFIC HYBRIDIZATION IN *BETA* L. I. EXPERIMENTAL SYNTHESIS AND ORIGIN OF *BETA TRIGYNA* W. et K. ($2n=54$)

By V. P. ZOSSIMOVICH

(Communicated by A. A. Sapehin, Member of the Academy, 15.VII.1938)

The genus *Beta* belongs to two provinces of species formation; both are confined to mountains.

The principal province of species formation of *Beta* L. is in Near Asia. From the section *Vulgares Tr.*, which probably is phylogenetically the most ancient one, was formed in this province the section *Corollinae Tr.*, which is represented by the species *B. macrorhiza* Stev., *B. lomatogona* F. et M., *B. trigyna* W. et K., *B. intermedia* Bunge and *B. nana* B. et H. The other province of species formation of *Beta* L. lies in the Canary Islands where, also probably from the section *Vulgares*, diverged the section *Patellares Tr.* containing the species *B. patellaris* Mog., *B. procumbens* Chr. Sm. and *B. Webbiana* Mog. The majority of species of *Beta* L. are characterized by a common chromosome number which is equal to eighteen ($2n=18$).

The species *B. trigyna* W. et K. from Crimea, Balkan peninsula and Hungary, on the other hand, proved to be represented by hexaploid populations ($2n=54$) ([2–5]).

The forms of wild beets collected by our expedition in Transcaucasia (1931) which were placed by taxonomists into the species *B. trigyna*, proved to be tetraploid ($2n=36$). The species *B. trigyna* thus proved to be represented by tetraploid and hexaploid populations which occupy different areas ([1–3]).

Parallel to the hybridization of the species *B. vulgaris* with the winter perennials of the section *Corollinae* we undertook cyclic interspecific crosses within this section.

Cytological analysis of the interspecific hybrids was made by N. Z. Zaikovskaja; it should have helped us in the solution of the problem of the origin of the species *B. trigyna*.

As a result of crosses made for the above purpose the following series of interspecific hybrids were obtained.

1. Triploid ($2n=27$) interspecific from the crosses ♀ *B. lomatogona* ($n=9$) × ♂ *B. trigyna* f. tetraploid ($n=18$).

2. Tetraploid ($2n=36$) hybrids ♀ *B. lomatogona* ($n=9$) × ♂ *B. trigyna* f. hexaploid ($n=27$).

3. Amphidiploid ($2n=54$) from the cross ♀ *B. lomatogona* ($2n=18$) × ♂ *B. trigyna f.* tetraploid ($2n=36$).

Triploid ($2n=27$) hybrids lt_{36} were sterile and showed in F_1 defective anthers and pollen.

The majority of flowers of F_1 pollinated with pollen of parental species remained sterile. When free pollinated with the pollen of parental species different plants lt_{36} showed differences as to fertility. Flowers of the triploid hybrids lt_{36} are similar in type to those of *B. lomatogona* and possess raised and slightly dehiscent sepals. In the tetraploid parental species the sepals are petaloid, lack greenish coloration along the keel and, later on, are wide open. The one-seededness of the fruit in *B. lomatogona* is recessive to the contrasting character of many-seededness in *B. trigyna* f. tetraploid. The hybrids lt_{36} are characterized by the presence of two- and three-flowered clots along those containing a single seed. The stems of the hybrids lt_{36} are more alike the sounded stems of *B. lomatogona* than octahedral stems of *B. trigyna* f. tetraploid.

In foliation of stems the hybrids lt_{36} approach the robust plants of *B. trigyna* f. tetraploid rather than *B. lomatogona* which is poor in stem leaves.

The tetraploid ($2n=36$) hybrids lt_{54} are fertile and typically possess normal pollen.

These hybrids show in F_1 intermediate characters between *B. lomatogona* and the hexaploid species *B. trigyna*. The fertility of these hybrids proves that in the haploid chromosome set of *B. trigyna* W. et K. ($n=27$) there is present a genome of 9 chromosomes which easily conjugate with the homologous chromosomes of the species *B. lomatogona*. It should be noted that tetraploid hybrids *trigyna*$\times$ *vulgaris* are sterile ([8]).

In type of flower these hybrids approach *B. lomatogona* and *B. trigyna* ($2n=54$). Stems of the lt_{54} are almost rounded and possess very small facets, similar to *B. lomatogona* and *B. trigyna* ($2n=54$). Foliation in the hybrids lt_{54} is weaker than in the hybrids lt_{36}.

Apart from these hybrids we have obtained also an amphidiploid, ♀ *B. lomatogona* ($2n=18$)$\times$ ♂ *B. trigyna* f. tetraploid ($2n=36$).

The amphidiploid possessed 54 chromosomes. It is a hybrid obtained in 1935. It bloomed only in 1937. In all the phenotypic characters our amphidiploid was indistinguishable from the plants of the hexaploid species *B. trigyna* W. et K.

The anthers and pollen were normal in the amphidiploid. Besides the amphidiploid $l_{18}t_{36}$ was as self-fertile as the species *B. trigyna* W. et K.

The species *B. lomatogona*, *B. macrorhiza* and the Transcaucasian tetraploid forms which were mistaken for the species *B. trigyna* are self-sterile when they bloom under the conditions of rather high temperature which is typical of the end of May and the beginning of June.

The analysis of the characters of the interspecific hybrids of this series of crossess, their comparison with each other and with the parental species unveils the ways of evolution, particularly the origin of one of the species, *B. trigyna* W. et K.

It may be taken for proved that the species *B. trigyna* W. et K. represents a natural amphidiploid and has originated from hybridization of the two previously existing species, *B. lomatogona* ($2n=18$) and *B. trigyna* f. tetraploid ($2n=36$).

As a result of interspecific hybridization we had to place the tetraploid forms of the species *B. trigyna* into a new species *Beta corolliflora* Zoss. ($2n=36$), which has an area of its own and all rights to be designated an independent species.

We shall present data which characterize the height of stems and the number of the latter in parental species and in the interspecific hybrids; all were planted at the same plot.

Parental species and interspecific hybrids	Chromosome number	Mean height of the stem of an individual in cm	Mean number of stems per individual	Genome structure
P. *Beta corolliflora* Zoss. (syn. *B. trigyna* f. tetraploidea—Transcaucasia)	36	112.2	12.0	*cccc*
P. *Beta trigyna* W. et K.	54	92.7	10.7	*llcccc*
P. *Beta lomatogona* F. et M.	18*	85.6	5.1	*ll*
F_1 ♀ *B. lomatogona* ($n=9$) × ♂ *B. corolliflora* ($n=18$)	27	97.4	12.2	*lcc*
F_1 ♀ *B. lomatogona* ($n=9$) × ♂ *B. trigyna* ($n=27$)	36	88.4	7.6	*llcc*
F_1 Amphidiploid ♀ *B. lomatogona* (2 $n=18$) × ♂ *B. corolliflora* (2 $n=36$)	54	97.0	8.0	*llcccc*

The tetraploid species *B. corolliflora* ($2n=36$) is characterized by the most robust development of stems and leaves.

The species *B. lomatogona* ($2n=18$) possesses rounded small-faceted, thin, short and slightly foliate stems. The species *B. trigyna* assumes an intermediate position between these species in the height and foliation of the stems. In the roundedness of the stem *B. trigyna* also approaches rather *B. lomatogona* than *B. corolliflora*. In the number of stems *B. corolliflora* is the richest species, *B. lomatogona* possesses the lowest number of stems. The species *B. trigyna* assumes an intermediate position as to the number of stems, though approaches *B. corolliflora*.

The triploid hybrids *lc* assume an intermediate position between the parental species as to the height of stems. In the number of stems the hybrids *lc* are close to the parental species. The tetraploid hybrids lt_{54} are intermediate between *B. lomatogona* and *B. trigyna* in the height of stems and in their number. These hybrids evidently possess the genome-structure *llcc*. One should emphasize their fertility in contrast to the tetraploid ($2n=36$) hybrids *B. trigyna* ($n=27$) × *B. vulgaris* ($n=9$) which are sterile. The height of stems in the amphidiploid $l_{18}c_{36}$ is very close to that in the triploid hybrids *lc*. The proportional duplication of the number of chromosomes of the parental species evidently had no positive effect on the productivity of the amphidiploid. The same is true also of the number of stems of the triploid hybrids and of the amphidiploid; this number approaches that of *B. corolliflora* and *B. trigyna*. The dominance of the rounded and small-faceted stems of *B. lomatogona* in triploid and tetraploid hybrids as well as in the amphidiploid explains the same character in the natural species *B. trigyna*.

The results of interspecific hybridization explain the causes of the smaller stem-height in the hexaploid species *B. trigyna* as compared with the tetraploid *B. corolliflora*.

The dominance of the morphological characters of the flower of *B. lomatogona* in hybrids *lc* and lt_{54}, the similarity of the floral type and of its

* Sirotina ([2,3]) has proved first that *B. lomatogona* has $2n=18$. Berg ([6]) regarded this species as a tetraploid ($2n=36$), and Scheibe ([7]) writes that there are in *B. lomatogona* two and three-flowered clots as well as single fruits.

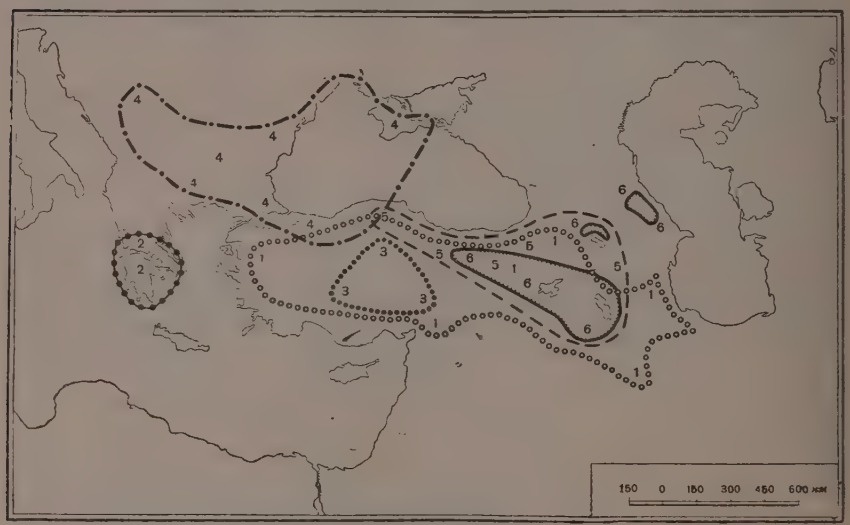
The genuine species *B. lomatogona* is characterized by constantly one-seeded fruits. It is possible that Berg obtained from Scheibe, instead of pure forms of *B. lomatogona*, natural tetraploid fertile hybrids lt_{54} which are phenotypically very close to *B. lomatogona* and possess, according to our data, two- and three-flowered clots and one-seeded fruits. The possibility of polyploidy is also not excluded in *B. lomatogona*.

generative organs in the amphidiploid and *B. trigyna* also confirm the origin of this species.

Both the amphidiploid and *B. trigyna* are characterized, beside the two- and three-flowered clots, also by the presence of single flowers and fruits, like those of the triploid and tetraploid hybrids of this series.

Sirotina (3), while studying cytologically the genus *Beta* L. and the section *Corollinae*, has established a remarkable regularity. It appeared

Geographical areas of distribution of wild species of *Beta* L.



Section *Corollinae Tr.*

. . . . . *1—Beta lomatogona* F. et M. (2*n*=18). Turkish Anatolia, Soviet Transcaucasia, Iran.

—·—·—·— *2—Beta nana* Boiss. et Heldr. (~~~~). Greece.

..... *3—Beta intermedia* Bunge (—). Central Anatolia.

——·—— *4—Beta trigyna* W. et K. (2*n*=54). North-west. Anatolia, Crimea, Balkan peninsula.

—— —— *5—Beta corolliflora* Zoss. (2*n*=36). Sov. Transcaucasia, Iran, East. a. centr. north Anatolia.

..... *6—Beta macrorhiza* Stev. (2*n*=18). East. Turk. Anatolia, Iran, Sov. Transcaucasia.

that species belonging to *Corollinae* are characterized by very similar sizes and ratios of the length of arms of the homologous chromosomes.

Sirotina succeeded in proving that the chromosomes of the basic genome of the tetraploid species *B. corolliflora* (syn. *B. trigyna* f. tetraploid) may be grouped in fours with regard to their morphology. Accordingly, in the hexaploid species *B. trigyna* the morphologically similar chromosomes may be grouped into sixes. Sirotina (3) was unable to distinguish in each group of six morphologically similar chromosomes two chromosomes belonging to *B. lomatogona* and four chromosomes belonging to *B. corroliflora*. She has, therefore, advanced a hypothesis of autotetraploid origin of our species *B. corolliflora* (syn. *B. trigyna* f. tetraploidea) and of hexaploid origin of the species *Beta trigyna* W. et K. Bleier (4) holds the same view on the origin of *Beta trigyna* (2*n*=54). Only Seitz (5) deduces from the

investigation of the reduction division in the hybrid *B. trigyna* × *B. vulgaris* that the species *B. trigyna* is an autohexaploid.

The results of interspecific hybridization clearly prove the allopolyploid origin of the species *B. trigyna* which is a natural amphidiploid.

Of great interest are the geographic areas of the species of the section *Corollinae* as shown in our digrammatic chart.

The species *B. lomatogona* grows under the arid conditions of the mountainous stepps of the Soviet Transcaucasia, of the western Iran and throughout the Turkish Anatolia at an altitude from 500 to 1500 m.

Populations of the newly discovered tetraploid species *B. corolliflora* grow in western Transcaucasia of the USSR, in western Iran and north-western Anatolia, however, in considerably more elevated and humid regions, than *B. lomatogona* (1500—2400 above the sea level).

In many localities these species are ecologically well differentiated. Along the Pontic chain of mountains in Asia Minor a narrow outgrowth of the area of *B. corolliflora* reaches the boundaries of the Kastamonu vilayet. The transgression of the areas of *B. lomatogona* and *B. corolliflora*, as may be seen from the diagram, is very significant. Natural hybridization could therefore occur in many localities, especially in the western part of the areas.

A. Scheibe ([7]) considered the tetraploid forms of *B. corolliflora* (*B. trigyna* f. *prealta* Koch.) to be the «genuine» species *B. trigyna*. The hexaploid populations of the species *B. trigyna* (f. *genuina* Koch.), which grow in the north-western Anatolia, the vilayets Zongudulak, Bolu, Kossaeli, Brussa), Scheibe took for «atypical».

The area of the hexaploid species *B. trigyna* lies beyond the areas of the two parental species *B. lomatogona* and *B. corolliflora*. The great drought-resistance of *B. lomatogona* in contrast to *B. corolliflora* enabled the amphidiploid species *B. trigyna* which originated from them to invade new provinces displaying conditions different from those existing in north-western Anatolia, in Crimea and on the Balkan peninsula as far as to Hungary.

Polyploid series are typical of many genera of old cultivated and wild plants from Near Asia. The majority of the parental species which gave birth to younger species with polyploid chromosome numbers still exist. Cytogenetic investigation of interspecific hybrids in a series of plants supplemented with ecogeography of the old and young species should, therefore, throw light upon their origin and bring us closer to a deeper understanding of divergence and of the ways of evolution.

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REDUCTION DIVISION IN INTERSPECIFIC HYBRIDS IN THE GENUS *BETA* L.

By N. E. ZAIKOVSKAJA

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The work on interspecific hybridization in the genus *Beta* L. is being conducted at the All-Union Research Institute of Sugar Industry by V. N. Zossimovich who has obtained a series of new interspecific hybrids. In the course of this work we have conducted cytological investigations, viz., determination of chromosome numbers in somatic cells and study of reduction division. The results of investigation of several interspecific hybrids are reported below.

1. *Beta lomatogona* F. et M. ($2n=18$) × *Beta trigyna* W. et K. ($2n=36$). Six F_1 plants of this cross were studied; their diploid chromosome number is equal to 27. In Table 1 is given the distribution of slides made of all the hybrids investigated, according to stage of reduction division. In the given hybrids about 27 chromatin clumps—prochromosomes—are formed at the leptonema-stage.

Table 1

Stage of division	Lepto-nema	Synap-sis	Diakine-sis II te-lophase	Tetrads	Pollen	Total
Number of slides	101	466	186	164	249	1166
Per cent	8.6	40	16	14.1	21.3	100

Then follows the stage of synapsis; according to Belar it is an artifact, and replaces the very susceptible to fixation zygonema- and pachinema-stages which are invisible in the beet plant. At the diplonema-stage 9 double and 9 single thickened threads are found; consequently 18 chromosomes out of 27 conjugate, while 9 remain univalent. At diakinesis (Table 2) 9 bivalents are seen, 1—2 (once 3) of the 9 univalents sometimes are attached to them to form trivalent compounds. Most often 18 elements are seen at the first metaphase, seldom 16—17 of them. The anaphase of the 1st division is irregular: bivalents form an equatorial plate, univalents are irregularly scattered along the spindle; however, they assemble at the poles first, most often 5—6 of them at the one pole and 4—3 at the other, at the moment when the bivalent just begin to disjoin. The division of the bivalents is irregular and is characterized by a asynchronous separation of the individual bivalents, by their lagging behind and by the two chromosomes of a common bivalent being directed towards one and the same pole. As a result of interkinesis (Table 3), aside from the two ordinary nuclei, accessory ones are sometimes formed, and single chromosomes are simply extruded into the cytoplasm. In one of the 6 plants of the cross investigated, reduction division ends at this point; after the nuclear division cytoplasm undergoes

Table 2

Chromosome Conjugation at Diakinesis

Origin of the hybrids	Trivalents	Bivalents	Univalents	Total number of chromosomes	Total number of elements	Number of cases
B. lomatogona × *B. trigyna*	—	9	9	18	27	4
	1	8	8	17	27	7
	2	7	7	16	27	2
	3	6	6	15	27	1
B. macrorhiza × *B. trigyna*	—	4	19	23	27	1
	—	6	15	21	27	1
	—	7	13	20	27	5
	1	6	12	19	27	2
	1	7	10	18	27	1
B. macrorhiza × *B. lomatogona*	—	4	10	14	18	2
	—	5	8	13	18	7
	—	6	6	12	18	3
	—	7	4	11	18	3
	—	7	4	11	18	1
	—	8	2	10	18	1
B. vulgaris × *B. lomatogona*	—	2	14	16	18	4
	—	3	12	15	18	3
	—	4	10	14	18	2
	—	5	8	13	18	1

division to form «diads» and pollen. In the remaining 5 plants a very irregular second meiotic division was observed; it was characterized by lagging behind of many chromosomes and formation of «bridges» of accesory nuclei or by elimination into the cytoplasm. The results of the 2nd division are represented in Table 4. The number of cells per «tetrad» varies from 2 to 6. The young pollen varies in size; at the beginning it is filled with plasm and during growth its content degenerates. In the mature anthers little pollen is generally found; under the microscope it turns out to be empty and shrivelled, filled normal pollen grains are of rare occurrence.

2. *B. macrorhiza* Stev. ($2n=18$)×*B. trigyna* W. et K. ($2n=36$). One plant from this cross with $2n=27$ chromosomes was studied. Reduction division is distinguished through the formation of a lower number of conjugating pairs (Table 2). The number of bivalents varies from 4 to 8, of univalents—from 10 to 19; in three cases one trivalent was observed. Simultaneous formation of «diad» and «tetrad» pollen, sometimes in a common pollen bag, is typical of the given hybrid. «Diads» consist of 2—4 cells, tetrads—of 2—6 (Tables 3 and 4); they may be distinguished only by their chromosome numbers. The total number in the former is equal to 27, in the latter—to 54. In mature anthers the pollen is of the kind described above; however, filled pollen grains are somewhat more numerous and they are larger in size. In some of the pollen grains first division is sometimes observed.

3. *B. macrorhiza* Stev. ($2n=18$)× *B. lomatogona* F. et M. ($2n=18$). Three plants from this cross, with the chromosome number $2n=18$, were investigated. Reduction division proceeds in the given hybrids more regularly. At diakinesis 4—8, most often 5—6, bivalents are formed (Table 2). At the anaphase of the 1st division single chromosomes lag; at interkinesis (Table 3) most often 7—8 or 10 chromosomes may be extruded into the cytoplasm. At the second meiotic division cases of lagging behind and of elimination of chromosomes into the cytoplasm as well as formation of accessory nuclei are observed more often. «Tetrads» commonly consist

Table 3

Distribution of Chromosomes at Diakinesis

Origin	First nucleus	Second nucleus	Accessory nuclei: Number of nuclei	Accessory nuclei: Number of chromosomes	Number of chromosomes in the plasm	Total number of chromosomes	Number of cases
B. lomatogona × *B. trigyna*	18	6	—	—	3	27	1
	17	10	—	—	—	27	2
	16	—	—	—	8	24	1
	14	13	—	—	—	27	1
	14	5	2	5	3	27	1
	13	13	—	—	1	27	1
	13	9	—	—	3	25	1
	12	11	—	—	4	27	2
	12	8	1	6	1	27	1
	11	—	—	—	15	26	1
B. macrorhiza × *B. trigyna*	18	—	—	—	7	25	1
	16	10	—	—	1	27	1
	14	13	—	—	—	27	1
	14	10	—	—	3	27	1
	14	—	—	—	12	26	1
	13	12	—	—	2	27	1
	12	12	1	3	—	27	1
	12	9	—	—	6	27	1
	12	5	—	—	10	27	1
	11	10	1	2	—	23	1
B. macrorhiza × *B. lomatogona*	10	8	—	—	—	18	2
	9	4	2	4	—	18	1
	9	9	—	—	—	18	6
	8	7	—	—	2	18	1
	10	8	—	—	2	18	1
B. vulgaris × *B. lomatogona*	7	2	—	—	9	18	1
	4	3	2	—	5	18	1
	3	3	1	3	9	18	1
	4	4	2	6	4	18	1
	5	4	2	6	3	18	1

of 4 cells, less often of 3 or 5; the number of chromosomes contained in them varies from 2 to 12 (Table 4), but 81.7 per cent of the tetrad cells contain 7—9 chromosomes. Young pollen grains differ in size and part of them afterwards degenerate. In some of the pollen grains (about 6 per cent) divisions and formation of two sperms may be seen. A little pollen is formed in mature anthers and the latter shed pollen slightly.

4. *B. vulgaris* L. (Uladovka, $2n=18$) × *B. lomatogona* F. et M. ($2n=18$). One plant from this cross with the chromosome number $2n=18$ was investigated. This cross is an interesting attempt of combining the properties of the cultivated beets with those of a wild species, which is distinguished by single seeded clots, cold-resistance and drought-resistance. The process of reduction division is disturbed in this hybrid in the highest degree. Most often 2—3 bivalents, seldom 4—5 (Table 2) are formed here at diakinesis. The first division is extremely irregular, chromosomes are scattered along the spindle. At interkinesis, instead of two, several small nuclei are formed; besides, many chromosomes lay isolated in the plasm (Table 3). The second division is similarly disturbed; the chromosome groups formed at interkinesis divide separately, and their number at the second telophase attains 10. The number of cells in «tetrads» varies from 2 to 6, and the chromosome number of these cells—from 3 to 17 (Table 4). Evidently several separate chromosome groups often get into a common cell which fact accounts for the formation of cells with high chromosome numbers (11—17). Very little pollen is contained in mature anthers.

Table 4

Chromosome Distribution after the 2nd Division

Origin	First nucleus	Second nucleus	Third nucleus	Fourth nucleus	Accessory nuclei: Number of nuclei	Accessory nuclei: Number of chromosomes	Number of chromosomes in the plasm	Total number of chromosomes	Number of cases
B. lomatogona × *B. trigyna*	27	—	—	—	—	—	26	53	1
	25	24	—	—	—	—	—	49	1
	19	14	12	4	—	—	3	52	1
	17	16	12	—	—	—	6	51	1
	15	12	12	7	—	—	2	48	1
	14	14	13	9	—	—	4	54	1
	14	12	12	11	—	—	3	52	1
	10	10	7	7	—	—	10	44	1
B. macrorhiza × *B. trigyna*	13	10	8	7	—	—	15	53	1
B. macrorhiza × *B. lomatogona*	12	10	5	2	—	—	1	30	1
	11	8	8	—	—	—	7	34	1
	10	9	9	8	—	—	—	36	2
	9	9	9	8	—	—	1	36	2
	9	9	9	8	—	—	—	35	1
	9	9	8	8	—	—	—	34	1
	9	9	9	—	—	—	9	36	1
	9	8	7	—	—	—	12	36	1
	9	9	8	7	1	3	—	36	1
	8	7	7	7	1	6	—	35	1
	9	8	8	7	1	4	—	36	1
B. vulgaris × *B. lomatogona*	17	16	—	—	—	—	3	36	9
	15	15	—	—	—	—	4	34	1
	15	9	7	5	—	—	—	36	1
	11	11	7	4	1	3	—	36	1
	11	11	5	5	—	—	6	36	1
	7	6	6	5	1	5	7	36	1

Conclusions. 1. Hybrids with 18 chromosomes occupy extreme positions as to the degree of disturbance of reduction division. Those having $2n=27$ assume intermediate positions.

2. Amount of pollen and its quality are in all cases strictly correlated with the degree of disturbance of the process of reduction division.

3. The «diad» pollen is better than that developing of «tetrads».

4. Two hypotheses on chromosome conjugation in hybrids whose father is *B. trigyna* ($2n=36$) may be advanced. First, that the chromosomes of the tetraploid parent conjugate with each other; secondly, that chromosomes of the mother conjugate with an equal number of the chromosomes of the father while the excessive chromosomes of the father remain univalent. The analysis of our data makes the second hypothesis more probable, for 1) in the tetraploid itself most often 18 bivalents are formed, 1—2 of which sometimes unite to form tetravalents, 2) a similar frequency of formation of higher compounds is observed in the two hybrids and 3) the type of chromosome conjugation is different in different hybrids which points to the conjugating chromosomes being different.

5. We have traced the conjugation of the chromosomes of *B. lomatogona* with three other species. The strongest affinity was shown by the chromosomes of *B. lomatogona* with those of *B. trigyna* (9 bivalents), a weaker affinity with those of *B. macrorhiza* (4—8), and the weakest—with *B. vulgaris* (2—4).

6. Chromosomes of *B. trigyna* display stronger affinity with those of *B. lomatagona* (9 bivalents) and a weaker one with *B. macrorhiza* (4—8).

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GENETICS

INHERITANCE OF YELLOW LEAF COLOUR IN *NICOTIANA TABACUM* L.

By P. V. MIKHAILOVA

(Communicated by A. A. Sapehin, Member of the Academy, 20.VII.1938)

The advantage of the yellow forms of tobacco plant for commercial growing is generally known and has been repeatedly emphasized by various writers. Yellowing of the tobacco leaves at the moment of harvest saves for the tobacco-grower much labour spent otherwise on seetheing and drying of leaves of green tobacco. The yellowing of leaves denotes, moreover, the correct moment of harvest which, as well known, is very important. We thus can see that the yellow colour of tobacco is of great economical importance, and the study of inheritance of the yellow colour represents, therefore, not only a theoretical interest but also has an undisputable practical value.

A series of works is known from the literature which are devoted to inheritance of the yellow leaf colour iu the genus *Nicotiana*. Lodewijks (7) has described two yellow forms of *N. tabacum*. Both of them when self-pollinated gave segregation into green and yellow with an excess of green plants. In the opinion of this writer his yellow tobacco-plants can exist only in heterozygous state, when selfed one form segregates in the ratio 75 per cent : : 25 per cent, the other—65 per cent : 35 per cent. V. Lubimenko and A. Palamarchuk(1), A. Palamarchuk and C. Jarkin(2), Kajanus(6)and finally Henika(5) have also described yellow forms in *N. tabacum*. The yellow-colour is controlled, according to their data, by two pairs of recessive genes. F_2 segregates in the ratio 15 : 1 with an obvious excess of green plants. It should be noted that some of the authors mentioned above (1, 2, 6) have not conclusively proved the dihybrid segregation of their tobaccos. The explanation of the interesting phenomenon of the excess of green plants as presented by Palamarchuk and Jarkin is also scarcely convincing. This explanation consists in the assumption that yellow seeds germinate poorer than green ones; the cases of their death in the soil are frequent.

It is of interest to mention the data on the inheritance of the yellow colour in *N. rustica* which was studied by Allard (3) and Bolsounov (4). Allard was dealing with a case of a simple monohybrid segregation with an excess of green plants, the latter being neglected by the author. Following Bolsounov his yellow *N. rustica*, on the other hand, depends on two recessive genes, and F_2 shows a segregation 15 : 0.9.

We see thus that all the above investigations lead to the conclusion that there are various types of inheritance of yellow colour; the question on the

reason for the excess of green plants remains, moreover, unclear. Similarly the writers do not indicate whether or not the plasm of the material organism takes part in the determination of the colour.

The purpose of our investigation was 1) to find out what type of inheritance of yellow colour we were dealing with; 2) what is responsible for the excess of green plants in the second generation; 3) whether or not the plasm of the maternal organism plays any determining rôle in the development of the one or the other colour; 4) what is the physiological mechanism of the realization of the character in question. The present investigation was started following a suggestion made by professor V. G. Rischkov who gave us also the seeds of the yellow variety Khodossevich and the green one—Samsoon. Segregation was observed on seedlings by the somewhat modified method of Henica.

Seeds were planted in Koch's dishes containing well wetted filter-paper and were left at room temperature and on light until the cotyledons reached maximum size, and the height of the individual seedlings attained 0.8—1.0 cm. The Koch's dishes were than moved to a thermostat in which a temperature of 32°C was kept, and on the 3rd day one could already see that the pure Khodossevichs lost the green colour and became quite yellow. The process of yellowing was usually completed on the sixth day. Following Henica, the appearance of the yellow colour in the seedlings of yellow tobacco was possible only when raised temperature and complete darkness acted together. We have observed, however, that when seeds of tobacco were germinated in a thermostat at 32°C in light, the seedlings of Samsoon were always green, and those of Khodossevich of gold-yellow colour; this gold-yellow colour remained permanently after the dishes were removed from the thermostat, while the seedlings of Samsoon received a deeper green coloration. The number of yellow and green seedlings were counted on the 6th day separately.

Our first generation was of two kinds as regards the plasm which was the result of reciprocal crosses. One of them possessed the plasm of Khodossevich, the other—that of Samsoon. The second generation was obtained from a back-cross of the F_1-hybrid plants to Khodossevich; here, as in F_1, we have made reciprocal crosses. The results of the F_2-segregation are summarized in Table 1.

Table 1

Summarized Data on the F_2 Segregation

Kind of cross	Experimental numbers			Expected numbers			χ^2 value
	Green	Yellow	Ratio	Green	Yellow	Ratio	$\chi^2=\Sigma\frac{d^2}{p}$
♀ Khodossevich × ♂ hybrid	17 895	5 089	3.51 : 1	17 238	5 746	3 : 1	100.151
♀ hybrid × ♂ Khodossevich	16 310	5 611	2.99 : 1	16 440	5 480	3 : 1	4.174

Note. While the discrepancy between the experimental and expected values of the first line is significant, it is insignificant in case of the second line.

We may see from Table 1 that in case the paternal plant is taken from variety Khodossevich and the mother plant is the hybrid, the discrepancy between the obtained and expected data is insignificant and may, therefore, be neglected; when summed up they give a ratio 2.99 : 1 which approximates the classical Mendel' s ratio.

The result is different when the paternal organism is of hybrid origin and gives pollen of four kinds, while the mother is of variety Khodossevich. Summarized data give a ratio 3.51 : 1 which significantly deviates from the theoretically expected one towards the excess of green forms.

While analyzing the results of segregation in F_2 we arrived first at the following conclusion (based upon the study of 44 905 plants): the inheritance of yellow colour is connected with nuclear heredity, the yellow colour being determined by 2 pairs of recessive factors. As the excess of green plants when the mother plant belonged to variety Khodossevich was great and the deviation of observed values from the expected ones was statistically significant, one may conclude that plasm had played no determining or leading rôle. The term «determining» should be emphasized here since there is no doubt that plasmic and nuclear elements most intimately interact in all cases. All counts were made by us under laboratory conditions; we find it erroneous, therefore, to follow Henica and Palamarchuk in explaining the excess of green plants by poor germination and death of yellow seedlings. In our experiments we could repeatedly see that both the seeds of green and yellow forms were late in germination. The germination percentage in Samsoon and Khodossevich, as shown in Table 2, is almost equal and somewhat higher in hybrids, so that no explanation of the increase of the number of green seedlings can be sought here.

Table 2

Germination of Seeds in Parental and Hybrid Forms of *N. tabacum*

Variety of tobacco-plant, hybrid combinations	Number of seeds planted	Number of seeds germinated	Germination percentage
Samsoon	800	676	84.5
Khodossevich	800	638	79.75
♀ Khod.×♂ (Khod.×Sams.)	800	720	90.0
♀ (Khod.×Sams.)×♂ Khod.	800	715	89.37
♀ Khod.×♂ (Sams.×Khod.)	800	708	88.5
♀ (Sams.×Khod.)×♂ Khod.	800	723	90.37

The excess of green seedlings could be also explained by the hypothesis that yellow forms are less viable and die at early stage of the zygote. No cytological investigations in this direction were conducted by us; however, indirect evidence—counting of seeds in capsules (Table 3)—shows that the cause of the excess should be sought elsewhere.

Table 3

Number of Seeds per Capsule in Parental Forms of *N. tabacum* and in Their Hybrids

Variety of tobacco-plant, hybrid combinations	Number of well filled seeds per capsule	Number of wrinkled seeds per capsule	Number of germinated wrinkled seeds
Samsoon	2 223	597	332
Khodossevich	2 241	586	340
♀ Khod.×♂ (Khod.×Sams.)	1 000	450	441
♀ (Khod.×Sams.)×♂ Khod.	1 804	347	253
♀ Khod.×♂ (Sams.×Khod.)	2 360	194	136
♀ (Sams.×Khod.)×♂ Khod.	2 680	207	180

One sole conclusion can be drawn from the foregoing, namely that the excess of green seedlings is conditioned by unevenness of the germination

of pollen; pollen bearing the factor for the green colour grows faster than that possessing the factor for the yellow colour. Final proof can be obtained only after application of the method of pistil-cutting; this work will be done now. As to the F_3 it was obtained through pollination of the green F_2 with pollen of variety Khodossevich. In case the colour is determined by two Mendelian factors the green F_2-plants, when pollinated with pollen of Khodossevich, should give segregation 3 : 1 and 1 : 1, the latter group being twice as numerous as the former. In our experiment out of 25 pollinated green plants 15 gave segregation closely approximating 1 : 1 and 10 plants—3 : 1. Instead of the ratio 2 : 1 we had 1.5 : 1. The deviation might be explained by the low number of plants pollinated.

It is absolutely evident that the study should not stop with determination of the mode of inheritance of the characters in question. The next step should be the demonstration of the physiological mechanism of realization of the given characters, establishing the mechanism of action of the hereditary factors, of the interaction between the hereditary bases of the organism and the external factors at various stages of development of the plant. This would bring us nearer to problems of purely practical kind. We have not touched upon this question here since it is the subject of a separate physiological work, as well as detailed cytological investigation of the yellow and green forms at various stages of development.

From the data obtained we are able to draw the following conclusions:

1. Inheritance of the yellow colour in tobacco plant is of dihybrid type, two recessive genes being involved.

2. Deficiency in yellow seedlings depends upon uneven germination of pollen of the hybrid plant (the green pollen germinates faster).

3. The main condition for decoloration of the seedlings of yellow tobacco is the temperature of 32°C.

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GENETICS

A CONTRIBUTION TO THE QUESTION ON THE RÔLE OF NUCLEINIC ACID IN CHROMOSOME CONJUGATION

By N. D. TARNAVSKY

(Communicated by A. A. Sapehin, Member of the Academy, 10.VII.1938)

The study of the biochemical processes and of their rôle in the alterations of the cell-nucleus during meiosis is both of theoretical and practical importance. This problem remains, however, as yet almost unexplored. While fragmentary knowledge of the biochemical structure of the cell-nucleus is available, the data on the nature of biochemical processes which take place during cell-division, on their connection with reorganization of the hereditary material and on their rôle in the realization of hereditary characters are very scarce.

While studying chemical organization of the cell-nucleus at various stages of its division, T. Caspersson has shown (1936) that the distribution of nucleinic acid undergoes characteristic changes during mitosis; he has shown also that at the stages preparatory to division the quantity of nucleinic acid is increased, while it decreases towards the end of division. He established thus the existence of a connection between the quantitative changes in nucleinic acid and the stages of cell-division. The question naturally arises whether there is no connection between the quantity of nucleinic acid and the process of chromosome conjugation which, as shown by Darlington (1932), appears to be a mere modification of the chromosome behaviour at ordinary mitosis. The data by Hershenson (in press) are also in favour of the existence of such a connection; he has shown that in *Drosophila* hyperploidy for inert chromosome regions (i. e. for regions where, following Caspersson, nucleinic acid is concentrated) lowers the frequency of crossing-over. In case there exists such a connection it should be possible to detect it by a change in crossing-over frequency. We have conducted an experiment with injection of nucleinic acid in order to study its influence upon crossing-over in the *X*-chromosomes of *Drosophila melanogaster*. The first results of this work are presented in this communication.

Method and Material. For the study of the influence of the nucleinic acid upon the crossing-over in the *X*-chromosome we have taken the line *sc v f car* and the normal one. Homozygous females *sc v f car* were crossed with males of the normal line. Heterozygous females were obtained from such a cross: they were selected in larval stage before preparation. Selected females were divided into four groups. Flies of the one group

were given injection of nucleinic acid* in saturated solution; this solution was taken for 100% concentrated. The second group was injected a 50% solution of nucleinic acid. The third group was injected with 25% solution of nucleinic acid. The flies of the fourth group received no nucleinic acid but were pierced with a needle; they served as control. Larvae of the females, which received injecton of nucleinic acid, as well as the controls, were placed separately into test-tubes containing medium where they underwent pupation. Females obtained from the pupae were crossed with males *sc v f car*, and the cross-over classes were then studied separately in the experimental groups and in the control. Nucleinic acid for injection was diluted with 0.5 per cent solution of NaOH. We have also conducted a study of the influence of the pure 0.5 per cent solution of NaOH upon the crossing-over in the *X*-chromosome, since nucleinic acid was injected in solution in 0.5 per cent NaOH. The method of this series of experiments is analogous to that used in the rest of experiments.

The condition of the experiment and control were identical: $t°$=24—25°C, yeast—medium, experimental and control crosses were made at the same time.

The Results of the Experiments and Conclusions. In the study of the influence of nucleinic acid and of the 0.5 per cent NaOH upon the crossing-over in the *X*-chromosome of *D. melanogaster* we have calculated the percentage of crossing-over in the regions *sc—v*; *v—f*; *f—car*. The results of the experiments are summarized in Tables 1 and 2.

Table 1

The Influence of ½% NaOH upon the Crossing-Over in the *X*-Chromosome

№	Interval	Per cent of chromosome crossing-over in the experiment with ½% NaOH	Per cent of chromosome crossing-over in the control	$M_1-M_2 \pm \sqrt{m_1^2+m_2^2}$	$\frac{M_1-M_2}{\sqrt{m_1^2+m_2^2}}$	Number of flies
1	*sc — v*	31.64±0.63	34.75±0.54	−3.11±0.83	3.8	In the experiment 4718
2	*v — f*	20.43±0.54	21.38±0.14	−0.95±0.70	1.35	
3	*f — car*	5.02±0.31	6.03±0.28	−1.01±0.42	2.4	In the control 6457

It may be seen from Table 1 that 0.5 per cent solution of NaOH causes a reduction of crossing-over percentage in the region *sc—v* as compared with the control. This reduction is statistically significant (coefficient 3.8). While there is a reduction of crossing-over percentage in the region *v—f*, the difference in the percentage of crossing-over in experiment as compared with the control is statistically insignificant (coefficient 1.35). The reduction of crossing-over in the region *f—car* is highly probable, though the difference is not wholly significant statistically (coefficient 2.4). The reducing influence of 0.5 per cent NaOH is thus the strongest at the ends of the *X*-chromosome, in the region *sc—v* at the left end, and in the region *f—car* at the right end; in the middle part of the *X*-chromosome, in the region *v—f*, no such influence was detected.

* We have used thymo-nucleinic acid of the *a*-form which was produced and kindly given to us by A. N. Belosersky (Moscow) to whom thanks are due.

Table 2

Influence of Nucleinic Acid upon the Crossing-Over in the *X*-Chromosome (saturated solution)

№	Interval	Per cent of chromosome crossing-over in the experiment with nucleinic acid	Per cent of chromosome crossing-over in the control	$M_1-M_2\pm\pm\sqrt{m_1^2+m_2^2}$	$\frac{M_1-M_2}{\sqrt{m_1^2+m_2^2}}$	Number of flies
			Saturated solution			
1	*sc—v*	34.41±1.09	32.72±1.14	1.69±1.58	1.06	In the experiment 1801
2	*v—f*	19.32±0.89	24.36±1.04	—5.04±1.37	—3.6	
3	*f—car*	4.00±0.44	7.05±0.54	—3.05±0.70	—4.35	In the control 1687
			50% solution			
1	*sc—v*	37.19±1.04	32.72±1.14	4.47±1.54	2.9	In the experiment 2113
2	*v—f*	19.97±0.83	24.36±1.04	4.39±1.33	—3.3	
3	*f—car*	4.21±0.43	7.05±0.54	—2.84±0.70	—4.05	In the control 1687
			25% solution			
1	*sc—v*	35.50±0.83	32.72±1.14	2.78±1.41	1.9	In the experiment 2991
2	*v—f*	21.09±0.24	24.36±1.04	—3.27±1.07	—3.05	
3	*f—car*	4.47±0.37	7.05±0.54	—2.58±0.66	—3.9	In the control 1687

The results of the nucleinic acid series show (Table 2) that nucleinic acid in saturated (100 per cent) and 25 per cent solutions does not influence crossing-over in the left part of the *X*-chromosome in the region *sc—v*, as the slight increase of the percentage of crossing-over which was detected in experiments proved to be statistically insignificant (coefficients 1.06 and 1.9). On the other hand, in the experiment with the 50% solution of nucleinic acid, the percentage of crossing-over in the region *sc—v* was increased as compared with the control. This increase is near to statistical significance (coefficient 2.9). The percentage of crossing-over in all the three experiments is lower in the regions *v—f* and *f—car* as compared with the control, the difference between the percentages of crossing-over in the experiments and in the control being everywhere statistically significant (coefficients in the region *v—f* being 3.6; 3.3; 3.05 and those in the region *f—car*—4.35; 4.05; 3.9).

From the above data it is evident that nucleinic acid influences the percentage of crossing-over in all the three concentrations. The effect is different from that produced by 0.5 per cent NaOH, viz., while 0.5 per cent NaOH causes a statistically significant reduction of crossing-over percentage in the left part of the *X*-chromosome in the region *sc—v*, in experiments with nucleinic acid in saturated and 25 per cent solution, although no statistically significant increase of the percentage of crossing-over is observed. There is absolutely no decrease either; in the experiment with

the 50 per cent solution, moreover, the increase of the crossing-over percentage as contrasted with the control is close to statistical significance. If we compare the results of the action of 0.5 per cent NaOH with that of all the three concentrations of nucleinic acid upon the crossing-over in the regions *v—f* and *f—car*, we may observe in the experiments with nucleinic acid in all the three concentrations in both regions a statistically significant increase of the crossing-over percentage in the experimental groups as compared with control, while in the experiment with 0.5 per cent NaOH there is no statistically significant reduction of crossing-over percentage in the region *v—f*, and in the region *f—car* it approaches statistical significance.

The fact that crossing-over percentage is reduced in experiments with nucleinic acid is very interesting and deserves attention and further study. It is characteristic that nucleinic acid causes reduction of the percentage of crossing-over in the region *v—f* and especially in the region *f—car*, i. e. exactly in those regions which are closer to the inert part of the *X*-chromosome. Starting with this one may draw a conclusion that injected nucleinic acid in all the three concentrations most strongly influences those parts which are the closest to the inert regions of the *X*-chromosome.

During investigations and while dividing flies into cross-over classes, phenotypic alterations were found in experiments with nucleinic acid: in 12 flies there was detected alteration of the type of the mutation bithorax and in 3 flies that of the type of the mutation Beadex. No such phenotypical alterations were discovered neither in the control, nor in the experiment with 0.5 per cent NaOH. Such specifically phenotypic alterations are very interesting and should be further investigated.

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AGROPHYSIOLOGY

NITROGEN SUPPLY TO SUNFLOWER ACCORDING TO DEVELOPMENT PERIODS

By N. A. KAŠHPOROV

(Communicated by D. N. Prianishnikov, Member of the Academy, 15.VII.1938)

Starting in our study of nitrogen supply to the sunflower, we took into account the prevailing opinion which considered the nitrogen fertilizers unfavourable to the development of this plant ([1,2]). Some data, however, available at that time on the periodic supply of nitrogen to the sugar beet and kok-saghyz (obtained in the laboratory of D. N. Prianishnikov) as well as our own observations on the absorption of nutrients by the sunflower([3]) prompted us to account for this opinion by that the problem of supplying the sunflower with nutrients, nitrogens in particular, had been approached without due regard to the time of development.

Proceeding from the above and from general biological points of nutrition we set ourselves the task of investigating the nitrogen supply at different periods with the aim to find out such a line of nitrogen supply levels («profile» of nitrogenous nutrition with respect to time) as should secure the highest yield of seed with a respective high content of fat in them.

The experiment was being carried on in a green-house at the laboratory of D. N. Prianishnikov in pots containing 9 kg of sand each. The plants (sunflower Saratovsky 0169) were supplied with tap water.

When the nutrition of plants is studied with reference to separate periods the success of such a study is to a great extent dependent upon a suitable division of the vegetation cycle into periods and upon appropriate estimation of the rations of separate nutritive elements supplied to the sunflower within these periods («supply levels»).

Table 1

№ of period	Period	Date	Duration	Supply levels			
				K	P	Mg and S	Fe
1	From planting till formation of 8—9 leaves	7.V—8.VI	32	1	1	1	1
2	Till the middle of heading . . .	8.VI—26.VI	18	4	2	4	3
3	Till the outset of blossoming . .	26.VI—7.VII	11	4	2	2	3
4	From blossoming till harvesting	7.VII—harvest	44 (55)	3	3	3	1

Note. Supply levels with respect to separate elements are expressed according to Hellriegel standard.

The literary data in hand(3) we have studied the levels of nitrogen supply against the background of other nutrients whose rations changed from period to period remaining constant within the same period.

The data referred to below show the division of the vegetation into separate periods with the respective levels of K, P, Mg, S and Fe supply.

The supply levels of nitrogen changed from period to period in different variants as follows:

Table 2

№ of variant	Nitrogen supply levels across the different periods				Note
	I	II	III	IV	
1	1	1	1	1	Supply levels are expressed according to Hellriegel standard for nitrogen
2	1	3	1/8	1/8	
3	1	3	3	1/8	
4	3 (from 23.V)	1/8	1/8	3	
5	1	3	3	3	

In changing the supply levels at the beginning of each period we had recourse to the method applied in the experiments with periodic supply carried out at the laboratory of D. N. Prianishnikov: the nutritive solution was sucked off the pots which afterwards were filled with water and the suction was repeated. In the laboratory this operation received the name «washing». Upon washing the pots were supplied with nutritive mixture, usually no more than one standard ration according to Hellriegel. The rest was introduced by equal rates through the tube and from above for the next 2—3 days in the morning and evening. Whenever we changed the nutritive solution two pots in each variant were put away.

The change of nitrogen supply levels across the periods resulted in both quantitative and qualitative change of the yield of sunflower (Table 3).

Table 3

№ of variant	Supply levels across periods				Air-dry weight in g		Kernel in %	% of moisture in the kernel	% of raw fat in the kernel	Yield of fat per 1 plant in g
	I	II	III	IV	Whole mass above ground	Seed				
1	1	1	1	1	85.2	27	63.2	9.0	48.7	7.56
2	1	3	1/8	1/8	80.4	18	56.7	8.2	52.5	4.92
3	1	3	3	1/8	91.4	29	62.3	8.0	52.7	8.76
4	3 (from 23/V)	1/8	1/8	3	40.6	7	63.8	11.4	41.9	1.65
5	1	3	3	3	114.7	29	61.5	8.9	46.4	7.54

The yield data bear evidence for that:

1. The change of nitrogen supply level from one to three rations of nitrogen according to Hellriegel standard affected but weakly the yield of seed and oil, provided this level was nearly constant in time; whereas changing with time it altered the yield and its quality considerably.

2. The highest yield of oil with a relatively high yield of seed and high content of oil in the kernel was obtained in the third nitrogen variant.

In this variant the plant throughout heading (29 days) enjoyed the highest level of nitrogen supply, this level having dropped markedly with outset of blossoming.

3. When the high level of nitrogen supply was prolonged over the period following the outset of blossoming (until harvesting) the yield of seed showed no change, but the fat ratio in the kernel dropped considerably (cf. variants 3 and 5).

4. When the level of nitrogen supply suffered an abrupt fall before the head had completed formation there was no notable change in the fat ratio of kernel, while the yield of seed and the kernel ratio dropped markedly (cf. variants 2 and 3).

5. A low level of nitrogen supply throughout heading followed by a sharp rise after the outset of blossoming lowered both the yield of seed and oil ratio in them and resulted in an exceedingly poor yield of oil per plant (cf. variant 4).

The data referred to above may be summed up as follows: a) the nitrogen supply level at the period of heading is in the first line responsible for the amount of seed yield in sunflower; the higher is the level during this period, or strictly speaking during its second half, the greater is the seed yield; b) the fat ratio in the seed is essentially determined by the level available for the period following the outset of blossoming: a high level during this period brings about a low fat ratio in the kernel.

Such an effect of the level of nitrogen supply changing across the periods is a final manifestation of its influence upon the biochemical processes in the plant. As the general trend and intensity of the transformations taking place in a plant are liable to a regular modification with age (N. P. Krenke), we have traced the dynamic of the «specific» (referred to one gram of mass) velocity with which the aboveground mass of the plant is being stored up, as well as of the rate of decrease of nitrogen ratio in the leaves (9 and 10, from below). This data after having been verified served

Table 4

Age Modifications in Sunflower

№ of variant	Nitrogen supply levels				Specific storing velocity of aboveground mass accumulated during					Rate of decrease of ratio of protein nitrogen in the leaves	
	I	II	III	IV	7.V—23.V	23.V—1.VI	1.VI—26.VI	26.VI—8.VII	8.VII—till harvesting	26.VI—8.VII	8.VII—11.VIII
Abs. 1	1	1	1	1	0.278	0.130	0.074	0.058	0.016	0.070	0.055
Relat.	—	—	—	—	100	100	100	100	100	100	100
2	1	3	1/3	1/3	100	100	99	117	81	142	155
3	1	3	3	1/3	100	100	99	124	93	79	155
4	3 (from 24.V)	1/3	1/3	3	100	90	98	69	113	12	9
5	1	3	3	3	100	100	99	124	119	79	108

as age indices. To facilitate the comparison of the variants the absolute values of the indices in variant 1 were assumed equal to 100, thus forming the units for the respective figures of other variants.

It may be seen from Table 4 that:

1. The nitrogen supply level exerts a strong influence upon trend and intensity of the transformations in sunflower.

2. The relative hightening of the nitrogen supply level calls forth a physiological renovation of the organism—increased storing velocity of the aboveground mass (some exceptions are traceable to other causes) and a slowing down of the decrease of protein nitrogen ratio in the leaves.

Comparing the data in Table 4 and 3 we can establish the following connection between the age of sunflower and the amount and quality of its yield:

1. The stronger the natural process of ageing is checked by the respective level of nitrogen supply (and by other means too) during the period previous to blossoming, the higher the yield of sunflower which may be expected.

2. The stronger the physiological ageing is expressed during the period following the outset of blossoming, the more ground we have to forecast a high content of fat in the kernel of sunflower.

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PLANT PHYSIOLOGY

EFFECT OF DAY LENGTH UPON GROWTH OF STEM PARENCHYMA CELLS IN SOYA

By G. M. PSAREV

(Communicated by A. A. Richter, Member of the Academy, 10.VII.1938)

A marked effect of day length on the growth functions of plants has been noted by the first workers in phoperiodism. Comparing the height of stems in plants given different photoperiods, Garner and Allard ([1],[2]) came to the conclusion that each plant is characterized by a peculiar day length most favourable to the growing up of its stem.

This view of American workers, however, about the character of the effect of day length on the growth functions of plants was based upon nothing but analysis of external quantitative-morphological characters such as general height of the stem and, sometimes, the average length of its internodes. It is a matter of regret that within following years no experimental work was done in this direction.

Deats ([3]), Pfeiffer ([4]) and Doroshenko ([5]) have concentrated their attention in the anatomic-morphological aspect of the problem and only partly touched upon more profound quantitative anatomical modifications which are induced in plants by the effect of day length. In the work of Doroshenko ([5]) we can find indication about size variation of epidermic cells in flax, barley and wheat under the influence of day and night periods.

Yet, the literature on photoperiodism so abundant in data reporting considerable changes in growth functions due to photoperiodic factor has paid no attention to the question as to the nature and character of these changes. This question, however, presents great theoretical interest if viewed with regard to present notions of the growth of cell and tissue, which functions are now put down to a special control apparatus in plant. To fill this gap, at least partly, the author was endeavouring to analyze the character of the photoperiodic response in some plants not only with regard to change of the development process but also growth functions.

The study of growth functions in the plants under experiment was carried on in two ways: first, by means of periodical measurements of the growth of plants within vegetative period as well as by measuring quantitative morphological characters linked with a definite age of plants; secondly, by measuring some anatomical elements in experimental plants.

The results reported here have been obtained by measuring the cells of the stem parenchyma in *Soya Illini* grown under different conditions as regards day length.

Method. As height of the stem and length of the internode changed regularly with day length, it was found expedient to register the length of cells in different internodes of the stem in plants which had been given different photoperiods. Six plants were taken for anatomical analysis; two of them were given 10-hour days; two, 18-hour days, while the last two plants were exposed to short day photoperiods during first five days and afterwards enjoyed an 18-hour day till the end of vegetation.

The material for anatomical investigation was taken from dry plants after their ripening. Its true shape was restored by soaking in hot water after which it was softened in a mixture of alcohol and glycerine.

To make out the differences in the length of cells from respective internodes, typical medullary cells were measured, taken from that part of the medulla which immediately followed the comparatively minute cells of the perimedullar zone. The measurements embraced several columns of these first rows of typical medullary cells. With regard to their state of preservation these cells are the most constant elements. The medulla was chosen for our experiment because 1) it presents the most simple object for such measurement, 2) in the course of plant's development its cells are less liable to casual change compared to those of some other tissue.

Measurements were done in two or three series of microsections. Each series comprised three or more slices, taken from the middle of each of the internodes under investigation. In each slice the cells were counted within the length of the ocular rule. The total number of measurements for an internode of the given series equalled ten.

I offer my heartiest thanks to N. F. Neuman who helped me in carrying out the microscopic investigations.

Results of Investigation. The plants tested can be characterized by the following figures of the height of the main stem and of average length of its internodes.

Table 1

Results of Morphological Description of Plants

№ of vessels	Plant periods	Height of stem	Number of internodes	Average length of internode	Note
1	Short (10-hour) day	15.9	10	1.59	
3	» » »	15.8	10	1.58	
91	Long (18-hour) day	226.7	32	7.08	
64	» » »	163.4	27	6.05	Injured by frost on 6. XI
95	Five short days followed by long days until end of experiment . .	228.0	36	6.33	
88	Five short days followed by long days until end of experiment . .	202.5	31	6.53	

Previous to anatomical analysis we measured the length of each internode in the stems of experimental plants. We give no results of these measurements here on account of the space available. They have shown the plants under test to differ greatly both with regard to their height and the length of internodes. The plants of the long and mixed (5 short days+long days) variants were 14 times higher and had a 4.2 times greater average length of internode than the plants treated with short days. The difference in the length of internode did not appear just from the bottom but was apparent only in the 5 or 6th internode. The lower internodes showed no marked difference as regards their length.

As may be seen from Table 2, the external mophological differences in the dimensions of the stem are linked with deeper modifications of the growth functions of cellular elements.

Table 2

Size of Cells of Stem Parenchyma in Soya, as Determined by Photoperiod

Photoperiod	Length of parenchyma cells in μ, measured in following internodes								
	1—3	5	6	7	8	9	10	15—16	17
Short day treatment	—	58.5	44.2	29.9	45.2	—	—	—	—
» » »	—	59.2	47.5	36.7	67.1	54.2	46.4	—	—
Long day treatment	41.8	—	—	—	—	—	—	63.3	—
» » »	—	—	—	—	—	—	—	—	70.9
Mixed day treatment (5 short days + long days)	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—

Photoperiod	Length of parenchyma cells in μ, measured in following internodes									
	18	23	24	25	28	29	30	31	32	33
Short day treatment	—	—	—	—	—	—	—	—	—	—
» » »	—	—	—	—	—	—	—	—	—	—
Long day treatment	—	—	—	—	97.4	—	102.7	—	—	—
» » »	—	81.2	76.5	79.6	—	—	—	—	—	—
Mixed day treatment (5 short days+long days)	75.0	—	—	—	—	—	—	84.8	108.3	81.2
	72.2	—	—	—	92.8	86.6	118.2	—	—	—

The length of the cells in the stem parenchyma of soya was notably changing with day length. A short (10-hour) day treatment produced a considerable diminution of cells from the lower to upper internode. A marked increase of the size of cells which begins with the 8th internode in both short day plants may possibly be due to an inner redistribution in the factor determining the growth of cells. In the long day plants, on the contrary, the length of cells is increasing gradually towards the top of the stem. The second plant of the long day variant (vessel 64) had their cells in the upper internodes increased to some lesser extent which should be put down to its being injured by the frost on September 6, i. e. 81—85 days before ripening. This circumstance has not only prevented a normal end of its vegetation but also seems to have prematurely stopped the growth functions in the upper internodes. In the mixed variant no decrease of the cells has been noted. The growing plants of this group increase their cells in the stem parenchyma in the same way as the long day plants do.

In all the plants tested we found the cells of the lower internodes to have the same size. We may then infer that the reverse alteration of the factor which determined the growth of the cells was increasing gradually as the plants developed under different conditions of light treatment, and that within certain range the quantitative alteration of the factor resulted in no immediate change of cells size.

The experimental data referred to enable us to draw following conclusions which should as yet be considered as preliminary.

1. Day length has a great influence upon the growth functions of soya plant (variety *Illini*). With reference to morphology this influence manifests itself either in marked depression of the upward growth of the stem which in plants treated with short (10-hour) day has less nodes and shorter internodes, or in pronounced promotion of this growth as in plants treated with long (18-hour) day which have more nodes and longer internodes.

2. By measuring the cells of the basic tissue of the stem from different internodes of the plant we were successful to establish a connection between the above external morphological modifications of the height due to the influence of day length and the differences in the size of cells. This connection was established both in plants which had suffered different treatment and in different intervals of one and the same plant.

3. The characteristic feature of these size modifications in the cells of the parenchyma of the stem is that in the short day plants the length of the cells is gradually decreasing from the lower to the upper internode (some exceptions may possibly be accounted for by an internal redistribution of the factor of growth) while the reverse is the case in long day plants where the cells increase their length in that direction. This holds true, however, only for the middle portion of the stem and upwards; the cells of the lower portion of the stem have approximately the same dimensions in all plants.

4. The gradual change of the cells' size reported here should be linked with some quantitative modification of the growth substance whose function is to control the growth of the cells in the plant.

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PHYTOPATHOLOGY

I. TOBACCO-MOSAIC VIRUS AS INFLUENCED BY MICROORGANISMS

By M. I. GOLDIN

(Communicated by N. I. Vavilov, Member of the Academy, 31.VII.1938)

In the spread of plant viruses in nature a large part is taken by the soil and vegetative remains. The question of viruses overwintering in the soil and vegetative remains and of their preservation in these media is of great practical importance.

Hoggan and Johnson (1) proved by experiments that a large part, if not the entire quantity of virus contained in dead plant tissue, lixiviates in the soil, and thus spreads over a considerable area. The further destiny of the virus which has permeated the soil is determined to a considerable extent by the character of biological processes in the soil. It is known that the majority of phytopathogenic bacteria succumb rapidly in unsterile ground. They are unable to stand the competition of saprophytes, and besides, are consumed by the protozoa. Thus, for example, according to our experiments, the culture of *B. mori*, when admixed in the amount of 5 billions to 100 g of soil, under sterile conditions, existed over two month, while in unsterile ground it perished as early as on the 3rd—5th day. *B. tumefaciens*, on the other hand, is able to persist in the soil throughout years.

In order to study the question of the spread of virus diseases in nature, it is very important to find out what might be the relations between viruses and microorganisms in pure cultures as well as in natural environment.

A detailed study of the character of influence exerted by microorganisms on plant viruses may furnish valuable material also for the knowledge of the nature of viruses. With this aim in view, various groups of microorganisms may be employed as special reagents. If, in particular, one should consider in accordance with Stanley's theory the virus of tobacco-mosaic as protein, it would be very important to find out what kind of bacteria would be able to decompose this protein, and under what conditions.

1. The Period of Preservation of Tobacco-Mosaic Virus under Different External Conditions. The influence of microorganisms on the stability of viruses in unsterile medium may be referrable to a number of conditions prevailing in the soil. Oxidation-reduction conditions present one of the most important factors.

The question of the effect of oxygen on plant has not been sufficiently treated. Johnson(2) introduced into the soil mosaic juice and dry leaves, and then, 2—4—5 months later, infected plants with this soil. From these experiments he arrived at the conclusion that the virus of tobacco-mosaic

may overwinter in the soil and that in grounds richer in moisture and with good aeration the virus looses ist activity sooner than in dry and solid grounds. We have carried out experiments on keeping tobacco-mosaic virus at temperature 25° under aerobic or anaerobic conditions (anaerobic conditions were brought about by means of alkaline solution of pyrogallol) with juice of mosaic tomatoes, filtered through filter-paper and with sterile filtrate (filtered through candles L3). The virus titre was calculated after Holmes' method, by inoculation of 2—3 leaves of *Nicotiana glutinosa* per each dilution. We have carried out over 10 series of experiments. Let us quote some of the examples (Table 1).

Table 1

Change in the Concentration of Tobacco-Mosaic Virus Kept under Aerobic or Anaerobic Conditions at Temperature 25°

		Number of necroses on leaves of *Nicotiana glutinosa* with dilution					
		Kept under aerobic conditions			Kept under anaerobic conditions		
		Undiluted	1:10	1:100	Undiluted	1:10	1:100
Virus juice unsterile	Exp. start. 14.IV. Test 1.VII	5,2,3	0,0,0	0,0,0	49,62,51	73,56,59	30,31,20
	Exp. start. 27.V. Test 27.VI	5,2,1	10,16,20	2,0	22,25	109,90,100	52,57,40
	Exp. start. 5.VII. Test 28.XI	17,10,7	1,1,3	—	42,27,35	33,43,49	—
Virus juice : filtrate (sterile)	Exp. start. 5.VII. Test 28.XI	0,0,0	0,0,0	—	10,19,23	7,2,9	—
	Exp. start. 26.VIII. Test 3.XII	5,1,0	0,0	—	41,36,53	2,3,10	—

The results of these experiments have shown that aerobic conditions promote inactivation of virus, kept not only in the presence of various microorganisms but also in sterile filtrate.

Attention should be paid to the somewhat peculiar character of various series of mosaic juice in regard to the stability of their titre. Different samples of mosaic juice, irrespective of the initial titre, retain their activity in different degrees. They lose it very rapidly within 1—1.5 months (even if kept at cold) while others remain active within a year and more. Thus, different samples of various juice are far from being always fit for comparison with each other.

2. Tests on Keeping Tobacco-Mosaic Virus in Pure Cultures as Related to Different Microorganisms. In 1926 Mulvania [3] for the first time experimented with the effect of various microorganisms on tobacco-mosaic virus. He performed altogether 2 tests, the results of which failed to be quite congruent. These two tests have led the above author to the conclusion that all microorganisms to a greater or lesser degree inactivate the virus.

Dunin[4] has discovered a very interesting fact. According to his experiments, when tobacco-mosaic virus is kept in the culture from *Macrospo-*

Table 2

Change in the Concentration of Tobacco-Mosaic Virus Kept under Aerobic or Anaerobic Conditions in Pure Cultures of Different Microorganisms at Temperature 25° (medium MP broth, pH=6.8)

		Dilutions	Filtrate: Number of necroses on leaves of *Nicotiana glutinosa*			
			No culture	+*B. mycoides*	+*B. coli comm.*	+*Torula kefiri*
Experiment started 27.V	Test 8.VI	Undiluted	37,22	46,57	26,20	1,0
	» »	1 : 10	9,70	37,20	13,19	2,3
	» »	1 : 100	4	4	2	0
	Test 15.VI	Undiluted	43,46	62,57	26,21	0,0
	» »	1 : 10	10,23	32,31	14,10	2,1
	» »	1 : 100	8	14	1,1	0
Exper. started 17.VI	Test 19.VI	1 : 10	32,36	55,35	23,18	9,6
	» »	1 : 100	14,13	14,11	6,8	0,0
	» »	1 : 1000	6,3	1,1	2,2	0,0
	Test 29.VI	Undiluted	30, 32, 36	44, 48, 52	20, 17, 14	2,1,0
	» »	1 : 10	32, 33, 28	31, 29, 26	20, 17	0, 0, 0
	» »	1 : 100	1, 8, 5	4, 7, 3	2, 3	0, 0

Culture	Exp. started 11.X, test 17.X. Number of necroses on leaves of *Nicotiana glutinosa*	
	Undiluted	1 : 10
Filtrate without culture	29, 35, 26	19, 10, 12
F+*Hefe E.*	28, 17, 20	14, 16, 12
F+*Sacch. cerev.*	3, 1, 0	12, 10, 14
F+*Torula kefiri*	3, 0, 3	2, 0, 1
F+*Shizosacch.* Pombe	37, 35, 31	9, 8, 5
F+*Debaram tyr.*	27, 22, 29	4, 8
F+*Sacchar. ellip.*	14, 10, 4	24, 37, 22
F+*B. coli comm.*	10, 16, 15	4, 9, 10
F+*B. mycoides* (strain 1)	21, 22, 18	34, 33, 39
F+*B. mycoides* (strain 2)	21, 22, 30	7, 3, 4

	Experiment started 21.VI		
	Test 25.VII		Test 2.I
	Undiluted	1 : 10	1 : 10
B. mycoides (strain 1)	19, 33, 50	10,2	8, 5, 6
B. mycoides (strain 2)	30, 45, 33	18,14	4, 5, 5
Control	45, 43, 40	3,2	9, 7, 5

Test of January 2 taken by the half-leaf method.

rium solani, the latter causes very sharp changes in the form-building properties of the tobacco-mosaic virus.

For the purpose of a more detailed investigation of the problem of the influence exerted by microorganisms on the tobacco-mosaic virus, we have undertaken experiments on keeping the virus in the presence of various microorganisms. As initial material the juice of mosaic tomatoes was used, which had been filtered first through a thin layer of asbestos and thereupon through candle L3. The test was performed in three variants. A culture of microorganisms was inoculated: a) in undiluted filtrate (pH=6.5—6.8); b) in a solution consisting of 2.7 cm³ MP broth (pH=7.2 and 6.0)+0.3 cm³ of filtrate; c) in a solution consisting of 3 cm³ of phosphates (pH=7.0)+1 cm³ of filtrate. All flasks were kept in a damp chamber at a temperature 25°. In the first series of tests as per variant a) with *B. mycoides* (isolated from the soil from mosaic diseased tomatoes), *B. coli comm.* and *Torula kefiri*, a sharp divergence became manifest in the behaviour of *B. mycoides* and *Torula kefiri*.

While in the culture *Torula kefiri* the virus lost its activity almost completely as early as on the 2nd day, the titre of the virus contained in the culture *B. mycoides* was not any lower than the control. *B. coli comm.* occupied an intermediate place. These tests were repeated a number of times in all three variants and yielded exactly the same results. After having established the inactivating action of the tested strain *Torula kefiri*, we further tried a number of other yeasts.

As may be seen from Table 2, the effect produced by different microorganisms on tobacco-mosaic virus is by far not alike.

In the presence of *B. mycoides* the virus not only remained active, but, in a number of cases, its titre proved even somewhat above the one in control. A rather favourable effect was also produced by the culture *Shizosacch.* Pombe: *Sacchar. cerev.*, pink yeast and particularly *Torula kefiri* (strain № 1) have markedly affected the activity of the virus in the course of a few day. The rest of the tested microorganisms exerted but a very mild influence on the stability of tobacco-mosaic virus*.

What is responsible for this effect of *Torula kefiri* and other microorganisms? Does it evolve directly in the process of development of the microorganism, or is the virus affected by products of metabolism?

Regular records of pH in cultures of these microorganisms show that the effect is not conditioned by changes in the concentration of pH.

It should be noted that the effect of *Torula kefiri* was displayed only under aerobic conditions. Under anaerobic conditions the tobacco-mosaic virus retained its titre in the culture of *Torula kefiri* over a month. Apart from the question as to whether the effect of microorganisms on the virus is positive or negative, there arises the following interesting question: if the virus of tobacco-mosaic is protein, is it possible it should be used by putrefactive bacteria? The first series of tests performed on *B. coli comm.*, *B. proteus vulgaris*, *B. prodigiosum*, *B. fluorescens liquifaciens* have shown that these microorganisms are unable to proliferate in a medium where the only possible source of energy consisted in Stanley's «crystalline» virus. The titre of the virus in these cultures was not to be distinguished from the control. These experiments are still in progress.

Conclusions

1. Sterile (filtrate) and unsterile virus juice loses its activity much sooner under aerobic than under anaerobic conditions.

* In the October issue of «Phytopathology» (1937) was published a paper by J. Johnson and J. Hoggan. The authors of this work affirm to have ascertained the inactivating effect of various bacteria and fungi on the tobacco-mosaic virus.

2. By far not all microorganisms developing in a medium containing tobacco-mosaic virus affect the activity of the latter. The titre of tobacco-mosaic virus, kept over 6 months in the culture *B. mycoides*, was not any lower than the control.

3. The putrefactive bacteria tested by us failed to decompose the «crystalline» tobacco-mosaic virus.

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II. ADSORPTION OF TOBACCO-MOSAIC VIRUS BY MICROORGANISMS

The question of adsorption of human and animal viruses as well as bacteriophages by various microbes has heen since long attracting the attention of investigators.

M. Rakieten and T. Rakieten (1) have establisted of late the very important fact of adsorption and inactivation of the staphylococcic bacteriophage by bacilli belonging to the group *B. subtilis*. As far as plant viruses are concerned, data are available only on the adsorption of plant viruses by various chemical substances. However, the question of adsorption of plant viruses by microbes may be of great importance, since it points to one of the possible ways of viruses spread in nature. We have made experiments on adsorption of tobacco-mosaic virus by microbes.

The present work was done with juice of tomatoes infected with tobacco-mosaic virus, as well as with «crystalline» tobacco-mosaic virus obtained by Stanley's method. Both samples were previously filtered through a sterile candle L3. The procedure was as follows: into flasks containing 1 cm^3 of filtrate each were added 2 cm^3 of phosphate compound with different pH values (pH=5.3; 5.8; 7.3; 8.3); whereupon into the flasks of the first series (pH=5.3; 5.8; 7.2; 8.3) was introduced a definite quantity of the microbe culture (according to the 2—3 billion standard). Into the second series with similar pH values another microbe was introduced, etc. The last 4 flasks (pH=5.3; 5.8; 7.3; 8.3) whereto no microbes were added served as controls.

All flasks were left for 2 hours in a thermostat at 37°, and then for 24 hours in an refrigerator. Thereafter all preparations were centrifuged for 5 minutes at a speed of 1000 r. p. m. The liquid obtained after centrifugation was drained to the utmost. At the bottom was left but a sediment consisting in the main of microbe bodies. Following this, into each flask were introduced 2 cm^3 of phosphate compound at corresponding pH value. Centrifugation and decantation were repeated 3—4 times. Thereupon the virus-content in sedimentation and in the last washing liquid was calculated. Prior to inoculation each sediment was mixed with 1 cm^3 of sterilized top-water (pH=6.8). Control (filtrate without admixture of microbes) was subjected to similar treatment. At the bottom of the control flask 0.1—0.2 cm^3 of filtrate was left.

The calculation of titre was made after Holmes' method by inoculation of 2—3 leaves of *Nicotiana glutinosa* per dilution. As seen from the table, tobacco-mosaic virus can be adsorbed by *B. mycoides* and *Shizosacchar.* Pombe. Acid medium is a condition favouring adsorption. When a buffer compound at pH=8.3 is introduced into flasks with filtrate, a flaky sediment settles, but just at this pH value no adsorption occurs, which testifies to the specific character of the adsorption observed by us.

Culture	Exper. 3.VIII (juice of tomatoes inoculated with tobacco-mosaic virus). Concentration of tobacco-mosaic virus after threefold centrifugation (number of necroses on leaves of *Nicotiana glutinosa*)							
	In supernatant liquid				In sediment			
	pH=5.3		pH=7.3		pH=5.3		pH=7.3	
	Undiluted	1:10	Undiluted	1:10	Undiluted	1:10	Undiluted	1:10
B. mycoides	0,0	0,0	0,0	0,0	16, 15, 6	2, 0, 1	0,0	0,0
Tor. kefiri (strain № 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Shizosacch. Pombe .	0,0	0,0	0,0	0,0	11, 4, 9	3, 2, 1	0,0	0,0
Control	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Experiment of 2.XI (Stanley's virus)								
B. mycoides	2,0	0,0	—	—	8,2	6,2	—	—
Tor. kefiri (strain № 1)	0,0	0,0	—	—	0,0	0,0	—	—
Shizosacch. Pombe .	0,0	0,0	—	—	2,0	2,1	—	—
Control	0,0	0,0	—	—	0,0	0,0	—	—
Experiment of 26.X								
	pH=5.3	pH=5.8	pH=7.3	pH=8.3	pH=5.3	pH=5.8	pH=7.3	pH=8.3
Shizosacch. Pombe .	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
B. mycoides	1,0	0,0	0,0	0,0	5,4	0,1	0,0	0,0
Tor. kefiri (strain № 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Tor. kefiri (strain № 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Sacch rom. ellip. .	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Pink yeast	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Control	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

It should be noted that the adsorbing agents proved to be microbes which, as shown by our experiments (2), have, unlike a number of other microorganisms, not promoted inactivation of tobacco-mosaic virus when thriving together with it.

Further on it is necessary to study the behaviour of tobacco-mosaic virus in a state of adsorption (relation to temperature, length of keeping, relation to antiseptics, etc.), as well as to make clear whether it is possible that virus should be adsorbed by killed microbes. Such experiments should determine the importances of the phenomenon observed by us in the spread and resistance of tobacco-mosaic virus under natural conditions of the soil.

Conclusions

Tobacco-mosaic virus may be adsorped by microorganisms (*B. mycoides* and others). Adsorption takes place in an acid medium and is of selective character.

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PHYTOPATHOLOGY

«HOLLOWNESS» IN CLARY SAGE (*SALVIA SCLAREA* L.)

By P. A. NESTERENKO and S. N. ZARITSKY

(Communicated by A. A. Richter, Member of the Academy, 28.VII.1938)

Among the principal essence bearing plants which are being cultivated in Crimea the clary sage is spread over the most considerable areas. The total area shared to this plant fluctuates between 2000 and 3000 hectars according to year, that is twice the area occupied by the rest of the plants from which essential oil is extracted—lavender and rose. With respect to the yield of sage oil the Crimean Autonomous Soviet Socialist Republic occupies the first place in the Union, its yearly produce being 3000 to 5000 kg, estimated with 2000 to 3000 thousand rubles.

The culture of sage has deeply taken root in the state and collective farm practice and offers a prospect of enormous success on account of its oil being of great importance to the perfumery and food industry. Aside from its high aromatic qualities the sage oil is one of the best fixatives when blended with other odoriferous substances.

For the last five-six years members of Crimean collective farms and agronomists have steadily been giving alarming reports about a notable waste of sage which makes its cultivation unprofitable after two years. Thus, the clary sage which is a perennial plant has turned into a biennial one in Crimea, with only one yield of flowers within the second year. It stands to reason that this phenomenon is unfavourable to the economy of state and collective farms, notably reducing their income. The devastating effect this plant is suffering is due to a disease which has been known in Crimean plantations for a long time and is there called «hollowness» of sage roots.

Cases are known when some plantations have been completely ravaged by the disease while the usual waste of plants after two years of cultivation makes 50—60% which is quite as much as to render the plantation unsuitable for working. The perished plants show huge hollow formations near the root crown which caused their exhaustion, rupture of vascular system and death. Numerous attempts to detect the causal agent responsible for the hollowness ended in failure until the investigation which is being reported here was carried out. The problem of protecting the sage plantation from this damage was more than once brought to attention by industrial bodies. The last time it was raised in a special report delivered at the All-Union Meeting of Essential Oil Industry Workers in 1937.

Yet as the causal agent of the disease had not been detected, the suggestions concerning the waste of sage and its control appeared to be nothing but most contradictory assumptions. The obscurity of responsible causes resulted in that the very fact of Crimean sage being a perennial plant was

put under question by many of the workers, who were inclined to set it aside from the perennial forms of Middle Asia. The cause of the devastation was sought in the biology of the Crimean sage which was supposed to be a mixture of biennial and perennial forms with the prevalence of the former.

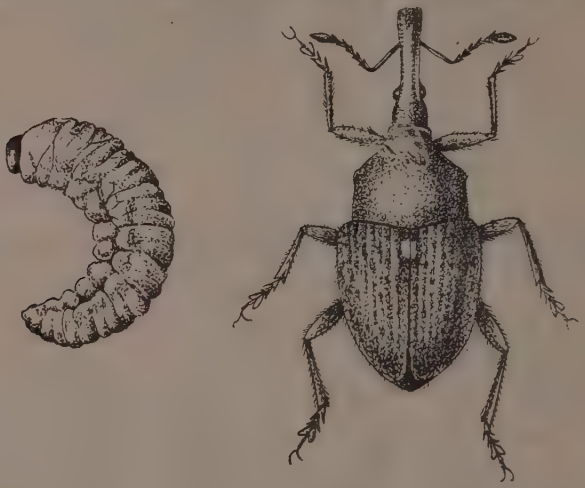

Fig. 1. *Phrydinchus topiarius* Germ. Larva and adult stage, magn. ×8

Such supposition had for its theoretical ground the possibility of the sage being liable to modification on the northern boundary of its habitat which runs over Crimea. This hypothesis, however, does not find support in the

Fig. 2. Left—formation of hollowness of the central root: right—central root preserved, while the plant perished all its sprouts being affected by larvae of *Phrydinchus topiarius* Germ.

practice, since cases have not seldom been reported from separate localities of Crimea when perennial plantations stood there out without or with but little waste whereas other plantations grown from the same seed fell into desolation after first flowering.

The suggestion that some insect might be responsible for the waste met objections because in some of the perished plants no hollows were

observed in the root and no parasite was detected. On that account the experiments set up by the All-Union Institute for Essential Oil Industry were directed towards discovering the bacterial and fungoid agents of hollowness, but so far as we are aware this investigation was not crowned with success.

More detailed observations throughout a year were needed to detect the parasite in the plants, affected with holowness, and account should thereby be taken of the biology of the plant. This work was started in the Department of Odoriferous Plants, Molotov (Nikitsky) Botanic Garden, in the spring of 1936.

As is known, at the crown of a sage root there are found every year in great numbers embryos of young sprouts which become floriferous the next summer;

Fig. 3. Young floriferous sprouts affected with hollowness. In the centre—larvae of *Phrydinchus topiarius* Germ.

after flowering these sprouts die away and new embryos appear in their place.

We have taken sprouts from wholly sound plants and made them root in a hothouse. Upon examination they proved to be infected with the larvae of *Phrydinchus topiarius* Germ. (subfam. *Centarrhynchinae*). The parasite has been identified after its adult stage by D. A. Ogloblin, of the Zoological Institute, Academy of Sciences of the USSR.

In different sets of the experiment the ratio of infected plants fluctuated within 15—47%; in the experiment of 1937 and 1938 the average infection of rooted sprouts was 26%. On the beginning of April the plants attacked by larvae were transported into open field; examined by the end of April they showed that the larvae had left their hollows. In some cases though very seldom we found single chrysalides at the entrance of the hollow. Examination of old plants in Nikitsky Botanic Garden done in the spring of 1938 revealed 61% of shrubs attacked by larvae of *Phrydinchus topiarius* Germ. 3 to 16 larvae were not rare in one shrub especially in its young floriferous sprouts. By the end of May the larvae left the hollows and went into soil to pupate.

134 hectars of sage commercial plantations in the state farm Krasnaya Rosa (Simferopol district) had 64% of plants attacked by hollowness; in the state farm Thälmann of the same district 44% of plants were found to be injured by the disease on the area of 90 hectars.

Further work will reveal the principal points in the biology of this harmful insect and thus afford essential support to elaboration of effective control measures. Still the observation now at hand allow us to point out some characteristic peculiarities of the parasite and the gravity of losses it is responsible for.

Phrydinchus topiarius Germ. lays eggs into the rosette of the plant in autumn, the emerging larvae biting their way into the plant to hibernate there and to form canals and hollows. In spring the larvae move in the soil for pupation leaving unprotected canals which afterwards grow into huge hollows. As sage is sown in late autumn (November) and the sprouts appear in spring, it is in the autumn of the next year that *Phrydinchus topiarius* Germ. lays his eggs in the young rosettes of the sprouts.

The autumn laying of eggs has been proved by an inspection of sage plantations in state farms Krasnaya Rosa and Thälmann where no hollows were found in the sprouts of 1938.

The ways eaten by larvae within the first year after infection being of insignificant dimensions, the first flowering of sage does not deviate substantially from its normal course. By the next year the hollows grow under the influence of fungous and bacterial diseases to such an extent that they cause rupture of the vessels in the root system and the death of plants. The death is probably accelerated by the formation of ice in hollows which leads to the rupture of the root crown.

If the larvae have bitten their ways up to the central root, hollowness is especially conspicuous.

Very often the young floriferous sprouts are alone infected by larvae which weakens their development—they form no flowers and ultimately die away. Such shrubs have no central hollows and this for a long time was adduced as an objection against entomological causes of the disease.

The damage due to *Phrydinchus topiarius* is enormous. Aside from direct death of hollowed plants which destroys a plantation completely after two years of cultivation, we face a considerable waste of flowers in the years of flowering owing to infection of young floriferous sprouts. The elimination of injurous effect of *Phrydinchus topiarius* will protract the life of sage plantations for several years, raise the yield of flowers considerably and thus make this culture more profitable.

Elaboration of control measures against *Phrydinchus topiarius* entails a more detailed biological study both of the insect and the plant itself. Agrotechnical measures (weeding and expecially a suitable crop rotation) seem to be the most promising in this respect.

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PHYTOPATHOLOGY

MOSAIC OF CULTIVATED CEREALS AND HOW IT IS COMMUNICATED IN NATURE

By K. S. SOUKHOV and A. M. VOVK

(Communicated by V. L. Komarov, Member of the Academy, 15.IX.1938)

In the summer of 1938 (May-August) we achieved our investigation of the mosaic disease in oats and other cereals, which had begun at Omsk in 1937. After having obtained some indirect evidence in support of this being a virus disease, probably due to insects, we set ourselves the task to determine the nature of the disease and to detect its responsible agent. With that aim in view we repeated the experiment of protecting the planted oats against insects by means of gauze insulators, which were adjusted to shelter the plots either immediately after sowing or upon the appearance of sprouts. As seen from Table 1, there were no signs of disease when a plot

Table 1

Experiments with Complete Insulation of Oats

№ of experiment	Date of sowing	Date of inspection	Insolated	Number of insulators	Number of plants under exper.	Number of diseased plants	% of diseased plants
Exper. № 1	12.V	10.VII	Direct after sowing	32	4548	—	—
Control without insulation	12.V	10.VII	—	—	5294	125	2.3
Experiment № 2 . .	30.V	6.VII	Direct after sowing	55	8137	—	—
Control without insulation	30.V	6.VII	—	—	23774	193	0.8
Total (experiment) .	—	—	Direct after sowing	87	12685	—	—
Total (control) . . .	—	—	—	—	29068	318	1.09
Experiment № 3 . .	30.V	7.VII	State of 1—2 leaves	2	310	2	0.64

was sheltered before sprouts had appeared. Whereas later sheltering resulted in an appearance of diseased plants. The most illuminative effect was obtained with incomplete insulation. In that case small gauze insulators were made use of, which were adjusted immediately upon sowing. While seedlings were growing we made small holes in the gauze of insulators and the tips of the leaves passed through them in the outside. We thus afforded the liberty for the insects to penetrate to the plants while the latter remained under the same conditions of growth as those established with usual insulators.

Table 2 shows that 8.5% of the perforated insulators included diseased plants (examination of plants was done 2 months after the beginning of the experiment).

Table 2

Experiments with Incomplete Insulation

Date of sowing	Date of inspection	Number of insulators	Number of plants inspected	Number of insulators with diseased plants	% of insulators with diseased plants	% of diseased plants
12.V	15.VII	364	8317	31	8.5	1.3

This proved the insects to be responsible for the disease and prompted us to start direct search for its carrier. As we had supposed the mosaic to be a virus disease, only sucking insects came into question. Thereby the lice and thrips were not suspicious in that respect since in our experiments of 1937 we failed to transmit the disease by their means, let alone that under the conditions prevailing at Omsk these insects are comparatively late in the oats which none the less are most badly injured by the mosaic if sown in early spring. Still we set up experiments with several species of lice which gave no positive results. Negative results too were obtained in the experiments with different species of bugs. At the same time we were carrying on experiments with *Delphax striatella*, *Cicadula sexnotata*, *Deltocephalus striatus*. These three species belong in the fauna of cultivated cereals. The method applied in the experiments was as follows. The insects were taken only from plants affected with the disease and moreover put on a diseased plant under gauze hood for 5—6 days. In case of virus disease such a measure insured infection. Afterwards the insects in different quantities were transferred to young seedlings of oats (2—3 leaflet stage) under large gauze insulators.

We have been successful in establishing that the mosaic of oats is transmitted by *Delphax striatella*. From Table 3 it may be seen that in a number of variants and in many separate experiments too *Delphax striatella* was invariably infecting the plants under the insulators and often gave a high ratio of diseased plants. The disease was accompanied by typical symptoms: dwarfishness, shrubbiness, green mosaic on the leaves and leaf sheaths, abnormal flowers, presence of *x*-bodies in the cells of leaf parenchyma. The period of incubation was found to be 7—9 days. Special experiments have proved that mosaic is transmitted by sexually mature males and females, and by larvae. That mosaic is more than a result of a mere zoological injure has been shown by the experiments with microinsulators. The insect placed in a microchamber was put on the emerging tip of a leaf of a gauze-sheltered plant. After 24 hours the microchamber with the insect was taken away, and on the 7th—9th day mosaic appeared on the leaves. The other symptoms became visible later on. Another evidence of the non-entomological character of the disease lies in the fact that it does not occur in the European part of the USSR though *Delphax striatella* is living there too.

Moreover we have obtained a direct evidence of the infectiousness of this disease. We let the diseased plants of oats grow together with the sound ones, this experiment being conducted under gauze insulators. 4% of sound plants became infected (out of 42 pairs of plants under experiment).

With regard to *Cicadula sexnotata* and *Deltocephalus striatus* our tests have shown that those species take no part in transmission of mosaic.

Thus, *Delphax striatella* has as yet bein proved to be the sole carrier of the disease.

By its means we succeeded in transmitting mosaic from oats to barley and conversely—from barley to oats, as well as from oats to millet. Aside

Table 3

Experiments with Infection of Oats by *Delphax striatella*

№ of insulators	Date of sowing	Date of inspect.	Number of insects	Number of plants inspect.	Number of diseased plants	Out of them		% of diseased plants
						Dwarfs	Mosaic non-dwarfs	
1	30.V	7.VIII	30	139	135	115	20	97.1
2	30.V	7.VIII	40	119	55	38	17	46.2
3	30.V	7.VIII	38	77	63	54	9	81.8
4	30.V	7.VIII	10	43	35	20	15	81.3
5	30.V	7.VIII	50	165	45	17	28	27.2
6	30.V	7.VIII	—	152	99	38	61	65.1
7—61	30.V	6.VIII	—	8137	—	—	—	—
62	30.VI	14.VIII	50	78	77	77	—	98.7
63	30.VI	14.VIII	80	92	87	41	46	94.5
64	30.VI	14.VIII	40	96	40	36	4	41.6
65	30.VI	14.VIII	37	98	65	55	10	66.3
66	30.VI	14.VIII	40	76	73	72	1	96.0
67	30.VI	14.VIII	30	60	58	53	5	96.6
68	30.VI	14.VIII	21	76	66	58	8	86.8
69	30.VI	14.VIII	30	59	43	40	3	72.9
70	30,VI	14.VIII	75	73	61	60	1	83.5
71	30.VI	14.VIII	15	67	28	27	1	41.7
72—75	30.VI	14.VIII	—	406	—	—	—	—
76	11.VII	14.VIII	20	66	46	45	1	69.6
77	11.VII	14.VIII	15	61	52	40	12	85.2
78	11.VII	14.VIII	23	57	50	45	5	87.7
79	11.VII	14.VIII	20	78	33	32	1	42.3
80	11.VII	14.VIII	20	86	35	33	2	40.6
81	11.VII	14.VIII	20	71	60	55	5	84.5
82	11.VII	14.VIII	30	61	19	17	2	31.1
83	11.VII	14.VIII	20	77	56	56	—	72.7
84	11.VII	14.VIII	80	75	71	71	—	94.6
85	11.VII	14.VIII	15	68	57	53	4	83.8
86	30.VI	14.VIII	4	71	26	22	4	36.6
87	30.VI	14.VIII	4	78	21	19	2	26.9
88—90	11.VI	14.VIII	—	205	—	—	—	—
	Total (Exper. with *Delphax striatella*)							
28	—	—	857	2319	1556	1289	267	67.1
	Total (Control)							
62	—	—	—	8748	—	—	—	—

from oats we have detected mosaic in rye, barley, millet and maize, which supports the data formerly reported by Pronicheva. Wheat seems also liable to infection.

Among perennial weeds we have tested, mosaic occurs in *Calamagrostis epigejos* Roth. This plant however seems to be very resistant to mosaic and only few infected individuals have been noted by us. The question of how the virus passes winter will constitute the subject of farther investigation. Yet we already now may suppose that it is the larva of *Delphax striatella* which keeps the virus throughout winter (*Delphax striatella*, in contradistinction to *Cicadula sexnotata* and *Deltocephalus striatus*, hibernates in the stage of a third age larva). Perennial weeds may possibly also play an important part in the conservation of the virus.

Cytological investigation of diseased plants, carried out by Sukhov, supported his former conclusion as to the presence of aciculate crystals

in the cells of the plants attacked by mosaic. At the same time new forms of giant crystals were discovered in the epidermic cells and closing cells of stomata. Those crystals usually have the shape of rings, eights, twisted braids, etc. They occur only in the cells of diseased plants and have never been found in sound ones. Both with regard to their dimensions and shape these formations are very similar to the protein crystals known in *Cactaceae*. Their nature of protein is supported by most simple microchemical reactions. They are readily soluble in water, are fixed by alcohol and Zenker solution, show a high affinity to acid fuchsin and give a positive Millon reaction. As regards their genesis they undergo a series of curious modifications; from homogeneous they turn into longitudinal fibres which sometimes break up into small aciculate crystals mentioned above. Giant protein crystals have also been found in the barley, millet and maize infected with mosaic. Upon fixation according to Zenker and

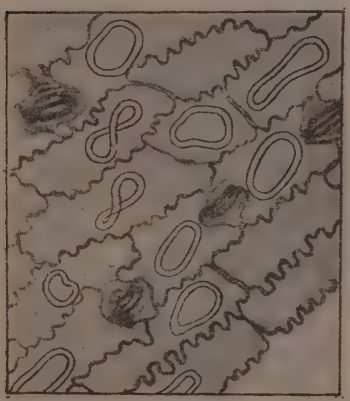

Fig. 1. Inclusions of protein in epidermic cells of maize infected with mosaic.

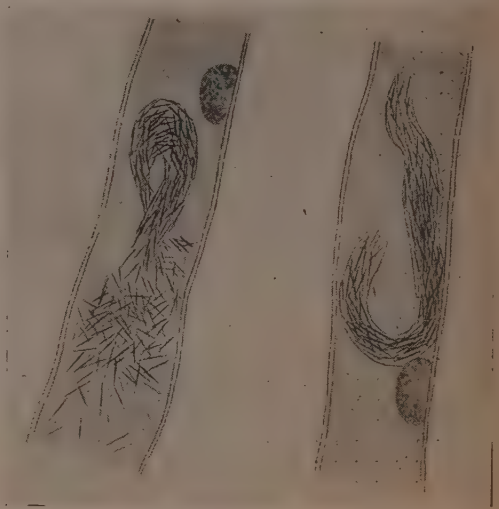

Fig. 2. Giant protein inclusions broken up into aciculate crystals.

farther treatment a deformation of crystals sets in, which we have observed in the preparations of Smirnova. As the crystals prove to be of the nature of protein, we may suppose them to constitute the virus in question.

Conclusions: 1. The mosaic of oats is a virus disease. 2. It is communicated by *Delphax striatella*. 3. The period of incubation lasts 7—9 days. 4. Rye, barley, millet and maize are also liable to this disease. 5. *Delphax striatella* can transmit the infection from one crop to another. 6. The cells of epidermis and closing cells of stomata in diseased plants contain giant protein crystals which are probably the virus of the oat mosaic.

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DEVELOPMENT MECHANICS

THE INFLUENCE OF EXCESSIVE PARATHYROID HORMONE ON THE DEVELOPMENT OF BONES

By S. A. IVANOVA

(Communicated by I. I. Schmalhausen, Member of the Academy, 10.VII.1938)

As it was shown by many authors, the process of development in the bone, as well as the changes observed in some distrophies, are under the hormonal control of the parathyroid gland. It has been established ([6], [4]), that fibrous ostitis, found in man, is the result of a hyperfunctional state of the parathyroid glands.

Many authors succeeded in producing a syndrom of fibrous ostitis in experimental animals by injecting an excessive amount of parathyroid hormone ([1], [2], [3], [5], [7], [8], [9], [10], [11]). Some of these authors established that following a fibrous ostitis, the activity of the osteoblasts was restored in the experimental animals, which was accompanied by the hypercalcimy of the bone (marmor disease) which means that the activity of the osteoblasts is also controlled by the parathyroid gland.

Studying the process of development in the bone it is impossible to omit the problem of its hormonal regulation. In spite of the fact that many phenomena have been studied in detail, it seems of interest and importance to study the hormonal control at the early stages of bone histogenesis. This question has so far not been dealt with by any of the authors.

Material and Methods. The experiments were performed on 1) pregnant female rats (the influence on the bones of the embryos was traced), 2) on young rats (2—9 days after birth), 3) on young guinea pigs (2 days after birth). The young animals were suckled till they grew able to feed. As controls we used animals of the same litter.

The parathyroid hormone (parathyreocrin of the factory of endocrinic preparations, Commissariat of Public Health, Moscow) was introduced subcutaneously. Control animals (rats) were injected with solution. The tibia was taken for microscopic examination.

1. Experiments on Pregnant Females. 14 pregnant rats were subcutaneously injected with parathyreocrin. 9 females received injections from the 6—7 till the 21st day of pregnancy. On the whole they received from 400 to 580 units of the hormone. 9 females were injected from the 13—15 till the 21st day of pregnancy. They received 45, 110 and 140 units per rat. Resorption of the embryos was observed in two females of the first group. Immediately after birth the tibiae of 51 new born were fixed for histological examination. For control 31 new born rats were taken from

females which had received no injections of the hormone. It has been established that no changes in the tibiae took place in the young female rats, as compared with the control ones. In the rats from the females of the first group given larger doses of the hormone, changes in the tibiae were established in three cases. They may be described as an osteoclastic resorption of the bone. It is necessary to note a great variety in the changes among the young rats of the same litter.

Thus, the rat № 44 gave birth to two young in one of which the osteoclastic resorption of the bone with a complete substitution of the resorbed trabeculae with fibrous tissue was very marked. In the second young rat the tibia was almost normal. The rat № 51 gave birth to 6 young, in which only in one or two there were more or less marked signs of resorption.

2. Experiments on Guinea Pigs. The guinea pigs at the age of two days were classed for the experiment into 3 groups.

The first group consisted of four experimental and two control animals. The experimental pigs were subcutaneously injected with 240 units of the hormone within 12 days. After that the animals were killed.

The second group consisted of two experimental and three control animals. The experimental animals were injected subcutaneously 700 units of the hormone within 65 days.

The third group consisted of two experimental and one control animals. The experimental animals were injected 1180 units of the hormone within 77 days.

A histological examination of the tibiae showed that all the animals of the first group, which had received 240 units of the hormone each, within 12 days, had a marked osteoclastic resorption of the bone trabeculae in the endochondral zone, with a substitution of the fibrous tissue. A great number of osteoclasts were present not only in the resorption zone of the trabeculae but often in the marrow too. All the animals of the second and third groups presented a different picture, namely, the resorption was absent. The epiphisar cartilage, the same as in control animals, consisted of 9—11 layers of cells. The zone of strongly developed trabeculae is 5—8 times higher than the cartilage zone. In this the bones of the experimental animals greatly differed from those of the control ones, in the latter the cartilage zone and the zone of trabeculae were almost equally developed.

Considerable accumulations of tissues of mesenchymal character, containing a large number of osteoblasts, were observed among the trabeculae in the bones of the experimental guinea pigs. Osteoclasts were present in a small number. In some cases slight hyperaemia was observed. The marrow has a normal fatty structure.

3. Experiments with Young Rats. Young rats born from normal females were taken for the experiment at the age of 2 to 9 days. The first group consisted of 5 experimental and 3 control animals. Two rats were subcutaneously injected 24 units of the hormone within 9 days, two were injected 86 units within the same period, while one rat was injected 106 units within 11 days.

The histological examination of the tibiae of these rats gave the following results. In two rats which received 24 units of the hormone within 9 days, we failed to establish any active bone resorption. In separate regions of endochondral ossification we found insignificant accumulations of osteoclasts. A different picture was observed in the bones of the rats which received 86 to 106 units of the hormone. The tibiae of these animals contained foci of intensive osteoclastic resorption with substitution of the osseous tissue by fibrous one. As opposed to control animals we found a large number of osteoclasts in the marrow.

The second group consisted of two experimental and one control animals. One of the experimental rats received 230 units of the hormone within 49 days, while the other received 300 units within 77 days.

The third group consisted of two experimental and two control animals. One of the experimental ones received 400 units of the hormone within 92 days, the other received 460 units within 100 days.

The fourth group consisted of four experimental and three control animals. Three experimental rats received 606 units of the hormone within 100 days and the fourth—620 units within 109 days.

Histological examination of the tibiae of the animals of the second, third and fourth groups showed that prolonged injection of the parathyroid hormone produced changes similar to those described in the experiments with guinea pigs. No resorption of bones was observed. The zone of vigorously developed bone trabeculae was 6—10 times higher than the cartilage zone. At the same time the zone of the trabeculae and the cartilage zone are approximately of the same size in the control animals. Considerable accumulation of tissues of mesenchymal character containing a large number of osteoblasts was observed among the trabeculae. Osteoclasts are rare. The marrow has a normal fatty structure.

Conclusions. Thus, our experiments permit of drawing the following conclusions:

1. The hormonal substance of the parathyroid gland, being injected to pregnant females, penetrates through the placenta and acts on the developing bone. We are the first to establish this fact. However, large doses are required to ensure a positive effect.

2. The preparation also affects the development of the bones in new born animals, the effect of a prolonged treatment differing from that of a short one.

3. A histological examination of bone-forming processes in the tibiae of embryos showed that the parathyroid hormone stimulates an intensive osteoclastic activity, resulting in a resorption of endochondral ossification and activation of the interosseous mesenchyma. Repeated injections produce a great number of trabeculae in the bone and osteoblasts are formed from activated mesenchyma.

4. The same resorption phenomena of the endochondral ossification are observed in some embryos at their birth.

5. The changes described by us show that phenomena like fibrous ostitis may be conditioned by an excessive introduction of the parathyroid hormone.

6. The action of the paratohormone (the hormonal substance of the parathyroid gland) on the endochondral process and on the activation of the mesenchyma presents some interest and requires further study.

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DEVELOPMENT MECHANICS

THE INDUCTIVE ACTION OF CARTILAGE IN VITRO

By A. W. RUMJANTZEW and L. F. BEREZKINA

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Gaillard (1) has demonstrated that it is possible to obtain in pure cultures of osteoblasts from the os frontale of the chicken embryo in experiments, continuing for a considerable period of time, the development of osseous tissue under the conditions of a consecutive addition of extracts from 4, 7, 10, 15, 18 days' old embryos to the culture and of a simultaneous growth retardation. In recently published experiments (2) we obtained the same results with mesenchyma. We have shown that it is possible to obtain the formation of typical cartilage from pure cultures of mesenchyma by adding to the medium some extract from developing bones of the same age. Since in the absence of the extract and in cultures of subcutaneous connective tissue, grown with the addition of this extract and with a simultaneous growth delay, there developed no cartilage, we were led to the conclusion that certain material principles are present in the extract of developing bone tissue, which activates the processes of the formation of the interstitial substance. However, such activation seems to be possible only in those cultivated tissues in which a capacity to form solid ground substance is preserved or present. Only mesenchyma and its ground substance can be considered as such. It may be presumed that in the ground fibrous substance of the mesenchyma, which we have shown(3) to become always organized in cultures, there are all integral parts necessary for the development of solid ground substance, while the activating substances, which aid or eventually direct and organize the process, are lacking. These substances are introduced from the developing bone in which they may be present in sufficient quantity. Having thus established the necessity of the presence of a determinate tissue for the development of the specific structures of ground substance, and confirmed it by experiments with a negative effect of bone extract on heart tissue and on subcutaneous connective tissue, we accordingly were obliged to express some doubts as to the possibility of obtaining the development of cartilage in various tissues, cultivated in vitro by introducing a bit of old cartilage into the culture, or, in other words, of obtaining the induction of cartilage by «old cartilage», as described by A. Fischer (4).

To verify his observations, important both theoretically and practically, we arranged the following experiments.

The material and methods used for this work were as follows. Bits of cartilage, taken from Meckel's cartilage of a hatched chicken, were implanted into pure cultures from the subcutaneous connective tissue of 15-day

old chicken embryo and into cultures from the heart muscle of 7-day old chicken embryo. Meckel's cartilage seems to us a most convenient material, since A. J. Studitsky* of our laboratory has shown that in vitro Meckel's cartilage taken from embryos at a late stage (beginning with the 12—13th day) does not grow. Implanted into a culture the fragment of the old cartilage was speedly overgrown on every side by the growing tissue of the culture and in the 2—3d passage after the implanting usually proved to occupy the center of the explants. Creating for the central zone the conditions of a retarded growth and causing by this a condensation of the tissue around the fragment of cartilage in the manner described in our previous paper, i. e. by cutting away the zone of growth alone in the course of further transfers to a fresh media,—we obtained finally after 8 passages (25 days) a compact, completely opaque fibrous mass of tissue around the cartilage, which reminded one of in vivo developing bone.

Results. Cultures with implanted cartilage and their control halves without implantation of cartilage were fixed between 14 and 25 days from the beginning of the experiment; sections were made of them and stained after various histological methods. The study of these cultures showed that cartilage had formed in none of them. We were able to establish with perfect preciseness that in the majority of cultures (with one exception) the implanted cartilage showed no signs of growth. Its cells degenerated, the interstitial substance suffered alterations (it liquefied a little and fibrils staining blue by anilin-blau appeared in it—the phenomenon of unmasking). This general process of the destruction of cartilage implanted into connective tissue cultures has been described in detail by Roulet[5]; in their main features our observations fully agree with his descriptions. However, we had the occasion to observe a type of the destruction and disintegration of cartilage in which fibrocytes played an active part. The swollen and somewhat unmasked interstitial substance in the course of the cultivation of cartilage with connective tissue begins to liquefy and fibrocytes begin to grow into the cartilage from the periphery. Penetrating into capsules containing remnants of disintegrating cartilage cells, the fibrocytes assume a round shape and thus simulate young cartilage cells. Around the bit of cartilage there usually developed a condensation of the tissue, and in the case of cultures from subcutaneous connective tissue collageneous fibres formed. We were unable to detect any specific influence of cartilage on the formation of fibrils. In examining our preparations we had the impression that fibrous structures developed the more, the deeper was the disintegration of cartilage. In one case a quite powerful knot of concentric fibres staining typically after Mallory formed in the center of the culture at the place earlier occupied by cartilage, which had disintegrated and liquefied.

Beyond the layer of fibres lay usually a dense zone of cells and beyond it the zone of growth. In cultures of muscle tissue also around the cartilage developed fibrils that were thicker than those in the control cultures, where by the 10th passage collageneous fibrils also develop, but always in thin and small fascicles. No cartilage tissue, nor even anything suggesting it was ever found by in any of the cultures. We must thus state that in the case of both subcutaneous connective tissue and in that of myocardium tissue we failed to obtain «induction of cartilage by cartilage». However, formation of fibrous structures and in particular formation of collageneous fibres was undoubtedly intensified.

No inductions were obtained in those cases either, in which well growing

* Unpublished data.

cartilage, taken from the eye capsule of the embryo, at the time when it consisted of cartilage, was implanted into tissue cultures. Cultivating it for 19 days with the myocardium tissue of a 6-day embryo, we observed growth of the cartilage by mytosis of its cells, degeneration of its interstitial substance, its penetration by fibrocytes growing into it, but never any formation of cartilage tissue in connection with the implanted bit. It is worth of noting that the formation of fibrils in the tissue of the experimental culture increased to such a degree, that growing muscle tissue resembled typical connective tissue.

If our observations are correct, and we are sure they are, then the experiments of Danini ([6]), made on animals, must be regarded as lacking conclusiveness. We often saw in sections of our cultures a quasi-formation of cartilage capsules around cells lying at some distance from the cartilage fragment and identical with what Danini described as precartilage stages. However, an attentive examination of the sections always showed these cells to be border cells of the cartilage fragment itself, which had assumed an irregular shape or may be already had it at the moment of implantation. In other words, one could without doubt be satisfied that these cells «keeping aloof» were stages of the disintegration of cartilage rather than of its formation.

By denying the possibility of obtaining cartilage from subcutaneous connective tissue or myocardium tissue, we deny eo ipso the possibility of redifferentiating a tissue, or the possibility of metaplasy, which is still advocated by some pathohistologists. The invariable preservation of their properties by tissues and their complexes is not only a postulate, but the general law of the development of organisms. We consider the explanation suggested by Danini ([7]), that some cells with a mesenchyma potency are preserved in subcutaneous connective tissue, as unconvincing.

Conclusions. Bits of Meckel's cartilage taken from new-hatched chickens and implanted into cultures from subcutaneous connective tissue of 15-day old chicken embryo or into those from myocardium tissue of 7-day chicken embryo do not grow, but doubtlessly intensify the formation of collageneous fibrils. This intensification of the formation of collagen becomes still more manifest when growing cartilage is introduced into cultures of myocardium tissue of a 6-day embryo. We were unable to obtain an inductive effect of cartilage on the tissues surrounding it, similar to the one described by A. Fisher, therefore we cannot assume cartilage to be the organizer of cartilage tissue. The intensification of the formation of fibrils in the presence of cartilage is easy to understand. Suffering disintegration and being dissolved in the ground substance of the culture, cartilage enriches this medium with products of disintegration of collagen, which probably represent the material utilized by the culture for an intensified formation of fibrils.

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